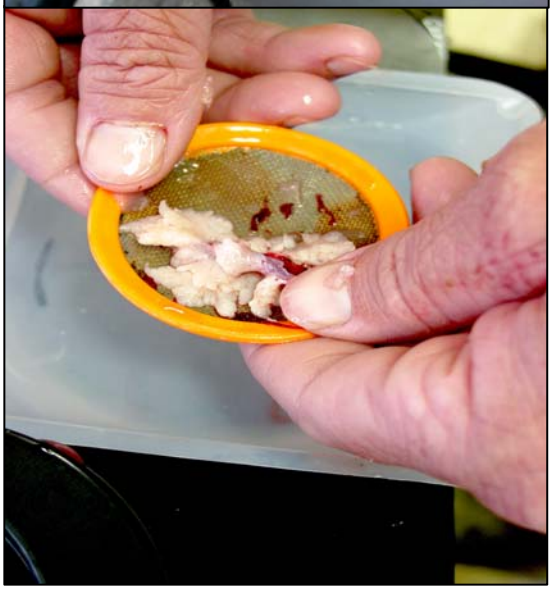

Producing Hybrid Catfish Fry:

Workshop Manual

USDA – ARS Catfish Genetics Research Unit

and the

Mississippi State Univeristy National Warmwater Aquaculture Center

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Acknowledgments

The workshop manual is organized into sections summarizing established techniques for hormone-induced spawning of catfish and highlights factors the authors consider important for production of hybrid catfish fry. Topic sections include text information, literature citations, a vendor/supplier list, forms, and copies of slide presentations used to highlight important points. Photographs have been included to illustrate important points and tables summarize important information. Supplementary materials in the form of SRAC publications have also been included.

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Introduction

Production of Eggs and Fingerlings

Production of hybrids between the channel catfish (*Ictalurus punctatus*) and the blue catfish (*Ictalurus furcatus*) was reported as early as 1966 (Giudice). Research to develop and refine methods for producing hybrids and to evaluate their performance for economically important traits has continued until present. The hybrid generally performs better than either parent species for several important production traits including survival, growth, disease resistance, and carcass yield (Giudice 1966, Yant et al. 1975, Dunham et al. 1983, Dunham et al. 1987, Dunham et al. 1990, Ramboux 1990, Wolters et al. 1996, Dunham and Argue 1998, Dunham and Brummett 1999, Chatakondi et al. 2000, Bosworth et al. 2004, Li et al. 2004). The hybrid produced by crossing a female channel catfish and blue male catfish is the mostly commonly produced hybrid and tends to be easier to produce and performs better than the blue catfish female x channel catfish male hybrid (Dunham et al.1982). Therefore, throughout this manual the term hybrid refers to the channel catfish female x blue catfish male hybrid.

The primary constraint to commercial production of the hybrid has been the lack of reliable, cost-effective methods for producing large quantities of fry needed for commercial catfish farming. However, refinements of techniques for producing hybrids and general superiority of hybrids relative to channel catfish have spurred renewed interest in use of hybrids for commercial production.

Traditional pond-spawning, which is effectively used to produce channel catfish fry, is ineffective for consistent, large-scale production of hybrid fry because of reproductive barriers (behavioral, physiological etc.) that prevent spawning between blue and channel catfish. Although there have been reports of successful spawning in brood ponds stocked with male blue catfish and female channel catfish, the occurrence of pond-spawning is very rare and not reliable for commercial hatchery production. Unless some future development dramatically improves the spawning rate in traditional brood ponds, production of hybrid fry will depend on the use of hormones to induce ovulation (final maturation and release of eggs) in females, manual 'stripping' of eggs, and manual fertilization of the eggs with blue catfish sperm (Tave and Smitherman 1982, Dunham et al. 2000).

Hormone-induced spawning is a commonly used technique for production of fry of many fish species and a variety of compounds (pituitary extract: common carp – CCP, or catfish CP, lutenizing hormone releasing hormone analog – LHRHa, human chorionic gonadotropin) have been successfully used for inducing ovulation in catfish. These compounds initiate the chain of events that lead to ovulation and release of eggs. The two most commonly used, and presumably most effective, compounds for inducing ovulation in channel catfish are CCP and LHRHa. Therefore the methods section of this manual will focus on the use of these two compounds.

Considerations in Using Hybrids in Growout Ponds

While the hybrid has several advantages in disease resistance, growth, and ease of catch, experienced hybrid producers report that there will have to be adjustments to the typical channel catfish production system to successfully raise hybrids. Due to the smaller size of the hybrid's head compared to a channel catfish, harvesting can be difficult if traditional mesh sizes are used (especially when using grading nets with larger than 1-inch mesh). Release of gilled fish often results in subsequent *Columnaris* infections, therefore most producers have used single-batch production for hybrids. Recently researchers at The University of Arkansas Pine Bluff have developed technology referred to as a 'panel-sock' which allows traditional sock grading of hybrid catfish and offers potential to raise hybrids in traditional multi-batch production scenarios.

Hybrids are also reported to congregate nearer the water surface at higher DO levels than channel catfish when needing aeration. Also, if improved growth and survival is realized, additional aeration will be required. Most hybrid growers recommend 3-4 hp of paddlewheel aeration per acre.

If possible contact a producer that has experience raising hybrids and consult with them on to get their perspectives on hybrid management. Although many aspects of channel catfish management apply to hybrids, there are differences associated with production and harvest that need to be considered.

This Workshop

The goal of this workshop is to present practical information and hands-on experience related to hormone-induced spawning and production of hybrid catfish fry. However, hormone-induced spawning of catfish is still part art and part science. The information supplied in the workshop provides basic information on techniques used for production of hybrid fry, but experience is the best way to learn how to produce hybrids. Start small, get a feel for the process and gain some experience, and alter the protocols and techniques as you see fit. Even people with substantial experience have variable results in producing hybrid fry, so don't be discouraged if your early results are less than what you expected. Also remember that the content emphasized in the workshop is based on the authors experience and knowledge. Other people may use variations in techniques and have different opinions on which issues are more or less important than those highlighted in this manual. You would be well served to gather information, advice, and opinions on the production of hybrid fry from as many sources as possible. We hope you find the workshop to be informative and enjoyable.

Producing Hybrid Catfish Fry

Hormone-induced production of hybrid catfish involves injecting female channel catfish with compounds to induce ovulation, determining the occurrence of ovulation, manually stripping eggs from females, and then fertilizing the eggs with blue catfish sperm. After fertilization the eggs can be hatched using procedures used to hatch channel catfish eggs.

Strategies for hormone-induced production of hybrids can be classified into two main categories: pair-spawning and group-spawning. In pair-spawning, a mature male and female channel catfish are placed in a tank together and the female is injected with hormone (sometimes the male is also injected but injection of males is probably not necessary). Typically a large aquarium or a tank with a window is used so the fish can be observed after injection. When the fish are observed engaging in spawning behavior (e.g. clenching – male and female lay head to tail with slight spasmodic contractions of the body and/or the presence of eggs in the tank) the female is removed and tranquilized. The female is then stripped of eggs and the eggs are fertilized with blue catfish sperm. Originally it was thought that the presence of the male and the behavioral interaction between the male and female were required for the ovulation process to proceed efficiently (Dupree et al. 1969) and pair-spawning was a commonly used method. However, research at Auburn University demonstrated that groups of females could be injected and held in tanks and ovulation would occur with or without male catfish being present (Dunham et al. 1998). Group-spawning allows larger numbers of females to be spawned in a more efficient manner and lends itself more readily to large scale production of hybrid fry than does pair-spawning. Therefore the techniques described in the manual are related to group-spawning of channel catfish females to produce hybrid fry.

Important factors for successful production of hybrid fry include: good broodstock quality, proper calculation and administration of hormone dosage, proper testes collection and sperm preparation, accurate determination of the time of ovulation in females, good stripping and fertilization techniques, and aggressive egg treatment. A general overview of the process followed by detailed sections on each of these topics follow.

Overview

This overview provides a summary of the group-spawning techniques used to produce hybrid catfish. It is difficult to spawn more than 80 to 100 fish per day with a 5 to 6 person crew. Split the fish into two groups of 40 to 50 each, with one group ‘scheduled’ to spawn a couple of hours before the other. Even though you may schedule the fish to spawn at a certain time, it rarely works out that way, but at least with two groups of fish scheduled to spawn at different times the workload will be spread out. Seine and select female catfish for spawning and handle to reduce stress. Select good quality females and transport them to your tank facility.

Hormone-induced spawning of catfish is generally a 3-day process. Pituitary and LHRHa typically require a dual injection process with an initial dose given the first day, a second dose given the second day, and the fish are spawned on the third day. Use the same injection schedule

for pituitary and LHRHa, specific information for pituitary and LHRHa is discussed later. Give the first injection the day females are seined. You can inject females as you take them off the transport tank or you can put females in tanks in the morning and then come back and inject them later. Weigh the females and inject with proper hormone dose (dosage is based on female weight so you will need to individually weigh the fish). Give the second injection between 7 and 10 a.m. the next day and females typically ovulate 24–30 hours later, although the time between the second injection and ovulation varies. Hold females in tank water temperatures of 25-27 °C (78-80 °F). On the third day, collect and prepare testes from blue males early in the morning, check to see if females have ovulated, and begin stripping eggs from females and fertilize eggs with blue catfish sperm.

- **Day 1** - Seine, select, and transport females to tanks. Weigh and inject females with first injection. Blue males can harvested and put in tanks on either Day 1 or Day 2, whichever is convenient.
- **Day 2** - Weigh and give females second injection.
- **Day 3** – Kill blue males, collect testes, and prepare sperm. Check females for ovulation, strip ovulated females, and fertilize eggs with blue catfish sperm.

Broodstock Care/selection

There are ongoing research projects to determine the effects of diet, strain of fish, and other broodstock management issues on the production of hybrid fry but results are not conclusive (Ligeon 1993, SRAC 17th Annual Progress Report 'Improving Reproductive Efficiency to Produce Channel x Blue Hybrid Catfish Fry' pp 61-76, included at back of manual). Therefore the same broodfish care and husbandry guidelines (feeding, water quality etc.) used to prepare catfish for pond spawning can be used to prepare catfish for hormone-induced spawning. One potential change between management of broodfish for pond vs. hormone-induced spawning is that higher stocking densities can probably be used and the proportion of males can be decreased if fish are only being used for hormone-induced spawning. However, using stocking densities as high as those used for food fish production or stocking only females in brood ponds intended for hormone-induced spawning is not suggested since the presence of male channel catfish in the ponds may play a beneficial role in the reproductive development of females.

It is important to realize that not all females will be selected for injection, one experienced hybrid producer believes only about 1/3 of females are actually injected. It is also important to have several ponds of broodfish because continual seining of the same pond likely will stress females and result in poor production.

Channel catfish females used for production of hybrid catfish fry should be 3 years old or older. It is generally believed that younger, smaller channel catfish females spawn later in the year and therefore it makes sense to use older, larger females for hybrid production early in the spawning season and younger females later in the season. Preferences for age/size of females used for hybrid production vary. A 5-8 pound fish may be the ideal size. Smaller fish don't produce many eggs per fish while stripping large numbers of big females can lead to fatigue. Information on broodfish management is available in SRAC publications included at the back of this manual: # 1802 (Channel Catfish Broodfish Management) and # 1803 (Channel Catfish Broodfish and Hatchery Management).

Perhaps the easiest way to determine when your female broodfish are reproductively ready for hormone-induced spawning is to put a few spawning cans in the pond and when you start to collect spawns you can assume some portion of the females are ready to spawn. Spawning activity in other ponds on your farm or on a neighboring farm is another good indicator that some of your fish are ready to spawn.

Stop feeding about 3 days before selecting females for spawning since one of best indicators of their suitability for spawning is a soft, full abdomen indicative of large, well developed ovaries (egg sacs). If the fish have been recently fed it is difficult to tell if the fullness of the abdomen is due to feed in the stomach or large ovaries. If the fish have been fed by mistake, wait a few days until the feed has cleared and then check them again. To select fish, hold females by the tail with their head hanging down and keep females that have a full, swollen belly. Another indicator of readiness for spawning is a red, swollen vent. However, we generally base our decisions on choosing a female for hybrid production on how full the belly looks since it is a rapid method to screen a larger number of fish. Fish with flat or only slightly swollen bellies are generally poor candidates for spawning and should not be used. The same pond can be screened again at a later date and some of the fish that were not ready the first time may be good candidates later in the spawning season. If you don't find enough good quality females to meet your needs, you should reduce the number of fish you plan on spawning or check another pond for better quality fish. It is a waste of money to inject poor quality females with hormone.

It is important to minimize stress during the selection procedure, avoid low oxygen and if the weather is warm try to seine in the mornings when the water is cooler. Don't pull the fish up and leave them concentrated in the seine for extended periods of time. Handle the fish quickly but carefully and load them onto transport tanks with adequate aeration, use pure oxygen when possible. Avoid rough handling, since it could result in damage to the ovary and lead to bleeding and clotting inside the ovary. Blood clots in the ovary make timing of ovulation and stripping females more difficult and will have a negative effect on spawning results.

Injecting females before or after normal pond spawning activity may reduce hybrid production. It seems logical that you would have better results with hormone-induced spawning if you are injecting fish when they are in prime spawning condition.

There has been some success using warm water wells to increase pond temperatures and accelerate the maturation process so that hybrid production can begin earlier than would be possible based on natural temperature cycles. However, the flow from warm water aquifers is generally limited and having a significant effect on the water temperature requires use of fairly small ponds. Research at LSU has suggested that 100 degree days above 21° C (~ 70° F) are required to bring catfish into spawning condition, that is equivalent to 25 days of 25° C (78° F) pond temperature: 100 degree days = [(25° C – 21° C) x 25 days] (see SRAC 17th Annual Progress Report). Hybrid striped bass producers frequently prolong their spawning season by holding white bass in cool water to maintain fish in spawning condition well past the normal spawning season. A similar approach might be useful for delaying spawning in catfish. However, any approach to accelerate or delay spawning by environmental manipulation will generally have additional costs associated with it.

Blue catfish males generally are not sexually mature until they are 4-5 years old (Graham 1999). Blue catfish can be sexed in the same manner as channel catfish, males have a pronounced genital papilla and the opening is rounded and females have a recessed papilla and the opening is slit like. Blue catfish males 'generally' have well developed testes during the same time frame that female channel catfish are ready to spawn. However, testes development varies widely among individuals and is difficult to predict an individual's testes development (and therefore its usefulness for hybrid production) based on the fish's external appearance or size. Using current methods, the status of testes development is not known until after the male has been killed and the testes have been surgically removed. We suggest harvesting about twice the number of males you think you will need for a round of spawning to insure sufficient testes/sperm for hybrid production. As a general rule of thumb, one blue male with well-developed testes will fertilize eggs from 8-10 average sized channel catfish females, so use 4-5 males with adequately developed testes to fertilize eggs from 32-50 females. Some producers weigh testes and use 1 g of testes per 250 to 500 ml of eggs. Masser and Dunham (1998, SRAC # 190, Production of Hybrid Catfish) suggest a lower ratio of 1 male for 3-5 females, or based on testes weight they recommend 0.5 g of testes be used to fertilize 100 ml of eggs (~ 100 grams or slightly less than a quarter pound of eggs). The ratio of blue males to channel females required depends on the size and development of the testes, the quality of the sperm, and the quantity of eggs produced by each female. It is important to use sufficient males/sperm to insure high fertility, but at the same time the cost and availability of blue catfish males requires that some attention be given to efficient use of blue catfish males. Development of a quick assay to identify the developmental status of blue catfish testes in live fish would be beneficial. Techniques for preparing sperm and checking sperm quality are described later in this manual. Unused males can be held in tanks and used for the next hybrid production cycle or returned to ponds.

Hormone Dosage and Administration

The two most commonly used compounds for inducing ovulation (final maturation and release of the eggs) in female channel catfish are pituitary extract- carp (CCP) or catfish (CP), and lutenizing hormone releasing hormone analog (LHRHa). Both compounds are effective and the opinions on which is best for hybrid production vary among users. We have used both successfully and both have advantages and disadvantages. LHRHa is a synthetic product and therefore it is a purified, consistent product. However, early in the spawning season the effective dose for LHRHa tends to be fairly high and therefore the cost is increased. In addition, the standard INAD (Investigational New Animal Drug) available from the U.S. Fish and Wildlife Service for LHRHa has a maximum limit of 100 ug/kg of female body weight, which is generally considered to be lower than the effective dose early in the spawning season. An INAD is required for use of either LHRHa or CCP (details related to INAD requirements are included later in the manual). Experience suggests that the time from injection to ovulation varies more among LHRH-injected fish than for CCP-injected fish. This can be an advantage or a disadvantage, it tends to increase the time you spend checking fish for ovulation but by spreading the timeframe for ovulation, you may be more accurate in determining ovulation for each individual fish. Additional information on reproductive biology and hormone-induced spawning of fish is available in SRAC publications included at the back of this manual: # 421 (Introduction to Hormone-Induced Spawning of Fish), # 422 (Capturing, Handling, Transporting, Injecting and Holding Brood Fish for Induced Spawning), # 424 (Hormonal Control of Reproduction in

Fish for Induced Spawning), # 425 (Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish), # 426 (Techniques for Taking and Fertilizing the Spawn of Fish).

Using CCP

CCP is dried, ground pituitary collected from carp. This organ lies directly beneath the brain. The main source of CCP in the U.S. is Stoller Fisheries in Iowa (see contact info in the appendix). Other vendors do sell CCP (for example Argent Laboratories), but most other vendors are supplied by Stoller Fisheries. The pituitary gland produces and stores gonadotropin hormones that play a key role in triggering ovulation. CCP is effective for inducing ovulation in a wide variety of fish species. Because it is collected from fish in an unpurified form, it is possible that the potency of CCP may vary from lot to lot. However, poor spawning results due to variation in CCP potency among lots have not been experienced. CCP appears to be more effective for inducing ovulation in catfish early in the spawning season than LHRH and CCP induced fish tend to have a more synchronous ovulation time than LHRHa fish.

The effective dosage for CCP induced ovulation of catfish is an initial (or priming) dose of 0.91 mg/lb (2 mg/kg) female body weight followed by a final (or resolving) dose of 3.64 mg/lb (8 mg/kg) female body weight. The timing of injection varies some among users, but typically the initial injection is given on day 1, the second injection is given 12-18 hours later after the first, and the fish ovulate 20-30 hours later. The time to ovulation varies among individual fish and therefore determining when ovulation has occurred is important for successful results (see next section). We typically give the initial injection sometime during the afternoon of the first day, give the second injection around 9 a.m. the next morning and then start checking for signs of ovulation the next morning around 7 a.m. However, these times are simply guidelines, the time between the last injection and ovulation varies among individuals, and it also tends to be longer early and shorter later in the spawning season, and longer at cooler holding water temperatures than at warmer temperatures.

CCP is typically sold in a dry powder form in 1 gram vials, it can be safely stored in the dry form in the dark at room temperatures for long periods (months - years). One gram of CCP is enough to inject about 220 lbs of female catfish. Prior to using CCP you should mix the powder with a physiological saline solution, use phosphate buffered saline (PBS). Contact information for vendors that sell premixed sterile PBS are listed in the appendix along with a recipe for PBS if you'd like to make your own. Mix the entire 1 gram vial of CCP at one time which eliminates the need to weigh and measure small amounts of CCP powder. If lesser amounts are needed, a fairly sensitive scale (one that measures to 0.01 g) will be needed.

Give CCP dosage in approximately 0.5 to 1.5 cc total volume. In order to make determining the injection volume straight forward, mix the concentrations so that 0.1 cc of CCP mixture is injected per lb of female body weight (a 5 lb female gets 0.5 cc and a 10 pound fish gets 1.0 cc). To do this, add the 1 gram of CCP powder to a tube containing 27.5 cc (=27.5 ml) of PBS = 36.4 mg CCP/cc, shake it up, and let it sit in the refrigerator for 1-2 hours. This lets some of the particulate matter settle out. The particulate can cause problems by plugging your syringe needle (use a needle with a 18 or 20 gauge needle, smaller needles will plug) and also seems to increase

the incidence of infections at the injection site. After the tube has sat for 1-2 hours most of the large particles will settle out, then use a syringe to pull 5.5 cc of the solution off the top and transfer this to a new tube and add 16.5 cc of fresh PBS to the 5.5 cc to get a total volume of 22 cc at a concentration of 9.1 mg CCP/cc. Label this tube as Day 1 injection (this is the solution used for the first injection). Shake up the original tube (36.4 mg/cc) and let it settle out again. Use a syringe and needle to pull off all the fluid you can, transfer to a new tube, and record the volume. It's OK if you get a little of the particulate, but try to avoid a lot of it. There is 22 cc of solution in the tube but you will probably only be able to draw out 19-20 cc. After you get what you can out, add enough fresh PBS back to the particulate so you can shake the tube and draw off enough to get a total of 22 cc. If you got 19 cc the first time, you want to get another 3 cc off the second time so the total is 22 cc. This solution is 36.4 mg CCP per cc, label it Day 2 injection (it is 4 times as concentrated as the Day 1 injection). Inject the females on the first day with the Day 1 injection solution at 0.1 cc/lb of body weight (= 0.91 mg CCP/lb) and inject fish on the second day with the Day 2 injection solution at 0.1 cc/lb of body weight (=3.64 mg CCP/lb). The mixed CCP can be stored in the refrigerator after mixing for a couple days or frozen. Reports indicate that freezing does not affect its potency, but avoid repeated freeze-thaw cycles.

The most commonly used injection site is the depression just behind the pelvic fin (see photo). Hold the fish by the tail with the head down and the fish can be quickly injected behind the pelvic fin. Use a 3 or 5 cc syringe with an 18 or 20 gauge needle for injecting CCP. A 3 or 5 cc syringe allows injection of fairly accurate amounts and will inject 30-50 lbs of fish. Using a needle with smaller diameter than 20 gauge with pituitary can result in frequent plugging. Move the fish that have been injected into another tank or section of the tank so you can keep them separate from the fish that still need to be injected.

Using LHRHa.

Use the same time schedule and technique to inject LHRHa. LHRHa is available from Syndel in Vancouver, British Columbia, Canada (see appendix for contact information). Other vendors sell LHRHa, but Syndel is the participating vendor listed on the INAD. LHRHa is sold in 1, 5, or 25 mg vials in a dry form. 5 mg is enough to inject ~ 110 lbs of female catfish at 45 ug/lb. The maximum LHRH dosage allowed on the standard INAD is 45 ug/lb of female body weight (100 ug/kg) and the total dose is split into a weaker initial and stronger final dose as with CCP.

Inject $\leq$ 1ml of hormone solution and so mix the concentration of hormone accordingly. Use a syringe to add 5 cc PBS to the vial and dissolve the powdered LHRHa, sometimes the powder will be stuck around the top of the vial so be sure to get it all dissolved. After it is dissolved, draw out the 5 cc, be careful because when you added it, there was probably some backpressure created. Add the 5 cc containing the 5 mg LHRHa to a clean tube and then add 8.8 cc more of PBS, now you have 13.8 cc of ~ 364 ug/cc solution (label this Day 2 injection). Mix this solution and use a syringe to draw off 2.8 cc of this solution and put it in another tube, add 8.2 cc of PBS to this tube to give a total volume of 11.0 cc of ~ 91 ug/cc (label this Day 1 injection). You should have 11.0 cc of Day 1 injection and 11.0 cc of Day 2 injection. Inject females on day 1 with the Day 1 solution at 0.1 cc per lb (= 9.1ug/lb). A 5 lb fish gets 0.5 cc and 10 lb fish

gets 1.0cc. Inject females on day 2 with the Day 2 solution at 0.1 cc per lb (= 36.4 ug/lb). A 5 lb fish gets 0.5 cc and 10 lb fish gets 1.0cc.

Testes Collection and Sperm Preparation

Improper handling and storage of testes/sperm can lead to poor or zero fertilization. As indicated earlier, it is difficult to estimate testes development from the external appearance of a blue male catfish so have approximately 2 times the number of blue males you think you will need seined up and ready for testes collection. Most people kill the blue catfish by a percussive blow to the head, this allows the fish to be eaten without concern about residual tranquilizer. In addition, there is a concern that if the fish are anesthetized, some of the anesthetic might get into the sperm preparation and negatively impact sperm motility. After the blue male has been killed, dry off the fishes skin well with a towel. It is very important to make sure that you do not get fresh water on the testes or in your sperm preparation because the sperm is activated by exposure to fresh water. After activation, the sperm only retains it motility and its ability to fertilize eggs for about 1 minute. Therefore, you need to make sure exposure to fresh water does not activate the sperm until you have mixed it with the eggs. Prior to fertilization the testes/sperm are kept in Hanks Buffered Salt Solution (HBSS), a solution that mimics the ionic concentration of the fish's body fluids and keeps the sperm in an inactive, but live state. If you activate the sperm during testes collection/preparation you can do everything else in the spawning process correctly and get little or no fertilization.

Place the male on its back and make an incision along the midline of the body cavity to expose the abdominal cavity. The testes are paired organs running from the vent toward the anterior part of the body cavity and lay along the upper wall of the body cavity. Well developed catfish testes are white and feathery in appearance, poorly developed testes are brownish pink and not as large. Remove the testes with a sharp knife, scalpel or by gently teasing them out with your fingers. The fish will bleed during this procedure and you can use a dry paper towel to blot up the blood. The blood should have ionic concentration similar to the testes so it should not activate the sperm, but try to minimize the amount of blood collected with the testes. Place the testes (or pieces of testes) in a container containing HBSS (the recipe for HBSS is included in the appendix, this recipe works well for me, other recipes are available and should be adequate). Use the disposable containers with lids available from Rubbermaid, Tupperware etc. They are cheap and the lid prevents water from being splashed into your HBSS. Rinse the testes in HBSS by moving it around in the solution to wash away blood and use your fingers or tweezers to remove blood clots. After the testes has been rinsed, move it to a container with about 1 cup (about 250 cc or ml) of HBSS. Place the testes in a metal sieve (commonly used for food preparation and available at Wal-Mart etc.) press the testes against the strainer with your thumb and fingers, this crushes the testes and releases the sperm into the HBSS (see photo). Continue crushing the testes until they are flat and most of the sperm have been released. Save the solution, it contains the sperm, The sperm solution will have a pinkish-white appearance. You can discard the tissue remaining in the sieve after crushing. Transfer the solution to a clean, waterproof container, either plastic or glass will work, and store the container on ice or in the refrigerator. Store the sperm solution on ice. Continue collecting testes from males until you feel you have sufficient sperm for your spawning requirements. Sperm solution from 4 or 5 blue males with decent testes development is sufficient to fertilize eggs from 35-40 fish, others may

recommend a lower number of males per female. Pool the sperm from 4-5 fish into your sperm solution container (Use 1 liter bottles which is about equivalent to 1 quart, just make sure the container is clean and dry!). Then increase the volume of the sperm solution to about 1.0-1.5 liters by adding more HBSS, not water. If you don't like the metric system, you can dilute the sperm solution to about 1 quart. Then estimate about how many spawns you think you will get, usually 75–90% of good quality females will ovulate, so if you injected 45-50 fish you will typically get 35-45 spawns and you can get an estimate of how much sperm to add to each spawn at fertilization. For example if you have 1 quart of sperm solution and think you will get 40 spawns, use 1/40 of a quart of sperm solution for each spawn. If you need more sperm you can collect additional testes and prepare it as you need it. Most producers use a large syringe to dispense sperm solution onto eggs. If females produce fewer spawns than expected you can use more sperm per spawn or you can store excess sperm solution refrigerated or on ice for at least 1 day with good results. See Christensen and Tiersch (1996) for information on catfish sperm refrigeration.

It is a good idea to check the motility of the sperm to insure you have good quality sperm prior to using it. The easiest method to check sperm quality is to examine it with a microscope. It does not require an expensive microscope, and you can probably borrow one from a local high school or veterinary clinic if you don't have access to one. Place a drop of the sperm solution on a microscope slide and add a slipcover, start with the low power objective and focus, then move up to a higher power. Under higher power the sperm should appear as small oval shaped dots and you may see a string like tail projecting from them. At this point the sperm should not be moving, you may see some movement of the fluid under the scope, this is called Brownian movement, and is simply the fluid under the cover slip moving. Don't worry if you see some sperm drifting across the slide as long as they are all drifting in the same direction. Then put a drop of water on the slide, then add a small drop of the sperm solution and add a cover slip. Now when you look in the microscope you should see the sperm moving quickly in many different directions. This is an indicator that the sperm quality is good and you prepared the sperm solution correctly. Not all the sperm will be motile, but if it looks like about 30-50% of them are moving, consider that to be good quality. Look fairly quickly, because the sperm will stop moving after about 1 minute. If there was a delay between adding the water and when you looked for motility and you don't see any movement, try another slide and look more quickly. If you still don't see any motile sperms you have problems and will likely have poor fertilization. You will need to check your HBSS preparation and make sure it is correct. Although motility may vary some from male to male and declines some during storage, if you have no motility it is generally an indicator that your HBSS was incorrectly made and the sperm were activated during collection and preparation.

Acquiring blue catfish males can be problematic since currently few people have large supplies of mature blue catfish available, but some producers are starting to grow blue catfish so availability should become less of an issue in the near future. Little information is available on the performance of hybrids produced by different blue catfish strains and we don't have any recommendation on what strain of blue catfish should be used.

Timing Ovulation

Proper timing of ovulation is critical to successful hybrid production. At ovulation, the eggs are released from the ovarian wall and if they are not removed the egg quality will begin to degrade. Therefore the key to successful strip-spawning of catfish (and other fish species) is to accurately determine when the female has ovulated. If you attempt to strip the female prior to ovulation, the eggs are not released and the yield of eggs and quality of eggs will be poor, if you wait until too long after ovulation, the egg yield may be good but the quality will be poor and fertilization will be low. In addition, catfish do not ovulate as synchronously as some fish species, meaning catfish do not ovulate and release all their eggs at one time. Therefore it can be difficult to determine if a fish is just starting to ovulate and you should wait until stripping her eggs or the process has proceeded to the point that the female is ready to be stripped. The best way to get good at determining when a female has ovulated and is ready to strip is through experience.

The easiest method to determine if ovulation has occurred in most fish species, including catfish, is to see if gentle pressure to the abdomen results in the flowing of eggs from the vent. Another method that has been used to help to determine if at least some portion of the female catfish in a group-spawning scenario have ovulated is to place a 4-6 foot long piece of 4 inch PVC in the bottom of the tank or vat downstream of the water flow. Tie a string and cork to the pipe so you can pull it up and occasionally take a look at it. On the morning you expect the fish to spawn, start looking at the pipe a 2-3 hours before you expect ovulation and examine it about every 30 minutes. At ovulation females will release some eggs into the water and some of the eggs will stick to the pipe. When you start to see eggs accumulating on the pipe it is a good indicator that you need to start checking the females for ovulation. To check females, gently crowd them and catch them with a dip-net. If you are right-handed, grasp the fish just ahead of the tail with your left-hand and hold her with her belly up so you can see the vent clearly. Use your right hand to gently squeeze the female's abdomen. Place the thumb on one side of her abdomen and forefinger on the other side and gently squeeze thumb and finger together as the hand moves down the abdomen towards the vent. Do this 3 or 4 times and if eggs are flowing from the vent the fish is ready for stripping. The amount of pressure required to get eggs to flow varies from fish to fish and relating the amount of pressure needed and how well the eggs flowed with a properly timed and stripped female can only really be learned through experience. Do not sedate fish when checking for ovulation. The fish that are not ready for spawning are put into another section of the raceway to be checked again about 2 hours later. You can sedate fish prior to checking them for ovulation but it slows the process and is probably not necessary. If you are having a difficult time deciding whether to strip or not or if the fish keeps contracting its muscles and won't relax, sedate it so you can get a better look.

Stripping and Fertilization

The fish that are determined to be ready for stripping are sedated by placing them in a container of aerated water with 100 ppm MS-222 (0.38 gram per gallon). When the fish is sedated, remove it from the anesthetic, dip it in fresh water to wash off the tranquilizer, and then dry the fish off with a towel. When the fish has been dried, grasp the fish at the base of the tail with your left hand and place the head of the fish in the crook of your right elbow with the fish belly down. The fishes head should be slightly elevated so gravity will help the eggs flow towards the

vent. Reach under the fish with your right hand and put your thumb on one side of the belly and fingers on the other side just ahead of the pelvic fins and gently squeeze as you slide your hand back toward the vent. Eggs should begin to flow out of the vent, continue this 'milking' process and move further up the abdomen as eggs begin to empty out of the rear portion of the ovary. Continue the process until the eggs stop flowing and/or the ovaries appear to be empty. When you strip a female a good indicator of proper timing is smooth flowing, yellowish green eggs, with few clumps or blood clots. A fish that does not flow well and has lots of egg clumps is usually a sign that ovulation was just starting or incomplete. Although putting these fish back in the tank and stripping them again later is an option, success with this practice has been poor. A fish that flows freely but the eggs come out somewhat stuck together or 'ropey' with a dull color and some whiteness is a good indicator that the eggs were ovulated earlier and have begun to degrade. Although some of these eggs may be viable, fertility is typically low and hatch is poor.

Catch the eggs in a plastic bucket or wash tub. Any container can be used, but it is important to remember that catfish eggs will stick to the container after fertilization if some type of lubricant is not put on the container surface. Various lubricants can be used (vegetable shortening, valve grease) but we find that non-stick cooking spray works well and is easy to use. Spray a small amount in the bucket and wiped it around and remove excess with a paper towel. Although some feel that cooking oil might inhibit fertilization by coating the eggs, there is no evidence that this occurs. After lubricating the container, put about 0.75-1.0 inch of HBSS in the bucket so the eggs go into the HBSS as they are stripped. After stripping the fish, put the eggs in a volumetric pitcher to get an estimate of egg numbers, then pour about 250 to 300 ml of eggs into a lubricant coated pie pan. You can make eggs masses larger but they get very thick and tend to have poor hatch because of reduced water flow and failure of treatments getting to eggs in the interior of a thick egg mass. If there is a lot of blood or egg clumps, rinse the eggs with HBSS and remove any large egg clumps or blood clots since these provide substrate for bacteria and fungal growth on eggs during hatching.

Use a fair amount of HBSS for egg washing. HBSS is cheap (~ 0.20 cents per gallon for the chemicals). For the sperm preparation, use HBSS mixed with distilled water, but for egg wash HBSS just use hatchery water (just be sure not to use a chlorinated source) and mix it up in 25-gallon batches in a large plastic garbage can. The first time filling the can with water, carefully measure out 25 gallons (or whatever volume you chose) and then mark the water level on the garbage can. This will allow you to fill the can to that mark and know it is 25 gallons. To make HBSS for egg washes, fill the can to the mark and then add the proper amount of chemicals and be sure to stir it well so the chemicals mix. You can drop an airstone in the water and that should provide adequate mixing and aerate the HBSS. Weigh out the chemicals for several batches of HBSS and store them in Ziploc® bags so all you have to do is add the water, then dump in the bag of pre-weighed chemicals and mix. Washing out the blood and removing egg clumps and other tissue improves fertility and hatch percentage. After the eggs have been rinsed, pour off the HBSS and the eggs are ready to be fertilized.

Store sperm solution on ice in a cooler. Add enough sperm for fertilization to the eggs and quickly stir it around, then fill the pan with about 1 inch of hatchery water and stir again quickly. At this point the sperm are activated and the eggs are fertilized. After a few minutes move the pans with eggs to a hatching trough with just enough water to cover the pans, carefully submerge

the pans and allow the water to flow over them and the eggs will water harden and stick together. When there is a break you can check the eggs and if they are stuck in a mass you can move them to the hatching troughs and hatch them as you would channel catfish.

Jar Hatching of Catfish Eggs

Many hatcheries use hatching jars to incubate eggs of various fish species and believe jar hatching improves hatch because dead and fungus infected eggs tend to be more buoyant and separate from the good eggs, preventing spread of the fungus. Jar hatching could improve hybrid catfish hatching success because of the high percentage of infertile eggs commonly encountered which result in spread of bacterial and fungal infections throughout the egg mass. However, the because catfish eggs are adhesive (stick together in a mass) they must be separated to allow jar hatching. We have tried several chemical treatments to eliminate adhesiveness of eggs but they were generally either ineffective or too harsh and damaged the egg. We recently determined that bromelain, a proteolytic enzyme derived from pineapples, is an effective and safe method for removing adhesiveness of catfish eggs for jar hatching. Hatch is generally improved, labor is reduced, treatments are reduced and hatchery space is reduced with the jar hatching technique. A brief description of the process follows.

We found that a 5' piece of acrylic tube in a 10" diameter works well for hatching catfish eggs. We put a flat irrigation cap on the bottom and inject water from a head tank down into the jar which up-wells from the bottom and back out the top (see diagram in slide handouts). We can put about 250,000 to 350,000 eggs in a jar with about 8 gallons per minute flow to keep the eggs suspended. The jars are easy to construct, cost is about $150.00 per jar. A few things we have learned about jar hatching is that you need to have a head tank with gravity feed to the jars to provide consistent flow. Gate valves are superior to ball valves for controlling water flow. If you have super-saturation in your water supply, tiny bubbles will form on the eggs and eventually float them out of the jar and into the drain. The bubble problem can be solved by putting an agitator in the head tank to degas the water. One potential problem with jar hatching is the potential for catastrophic failure. If you have 350,000 eggs in a jar and the water flow stops for even a short period, oxygen will be depleted and the embryos will die, we are currently working to develop emergency aeration to fix this problem.

We mix bromelain at 4000 gel dissolving units per liter of hatchery water. You can purchase it as a tablet from health food stores on line or as bulk powder. Gel dissolving units or GDU is a measure of the activity of the enzyme. If you use tablets, crush them and mix it up the morning before use because it does not dissolve easily. Mix it up fresh daily. Mix this solution about 1 to 1 with fertilized eggs, about 3 to 5 minutes after fertilization. Treat the eggs prior to them starting to stick, the treatment does not work well to separate eggs after they have stuck together but it does prevent them from sticking. Stir eggs with bromelain for a couple minutes and then add to hatching jar by pouring into a funnel connect to a pipe discharging near the bottom of the jar. Treatment cost is about 25 cents per spawn and the solution can be reused several times.

LHRHa Induced Spawning of Channel Catfish Suspended in Bags

Weighing and placing individual fish in bags as opposed to communally stocking them in tanks.

Black spawning bags are 24" X 36" with 5/16 inch mesh. The process of maturation and ovulation in channel catfish is not synchronized. Hence, there are no known methods to predict ovulation in hormone induced fish. Typically at the end of the latency period, hormone induced fish are repeatedly observed for signs of ovulation. This repeated handling and stress hinders the prospects of ovulation and also yield lower quality of eggs resulting in lower performance. Placing of individual fish in a 8 mm mesh nylon bag (2' x 2') is an option to overcome the problem. The bags with the fish are suspended 6 inches below the surface in a flow-through concrete tank. It is advisable to maintain a minimum distance of 1 foot from each bag on all sides. As individual bag can be marked or clipped at the time of first injection and need not be weighted during the second injection. At the end of the latency period, the bag need to be slightly lifted above the water to see the presence of expressed eggs along the seam – indicative of the fish's readiness to ovulate for hand stripping.

Hormone preparation (stock solution, 1st and 2nd injection): Hormone solution preparation: Make sure to keep LHRHa bottle in freezer (-20° C) to maintain its potency. As weighing this hormone needs sophistication, it is recommended to mix the hormone with appropriate volume of 0.85% saline and aliquot the desired volume for 1st and 2nd injections. (Mixing 8.5 g of salt in 1 L of distilled water or 32.3 g of salt in 1 gallon of distilled water gives you 0.85% saline solution).

LHRHa induced ovulation in catfish procedure is a 3-day process: Day 1) Brood fish selection – seine, select, transport and hold them in indoor facilities, 1st hormone injection (evening). Day 2) 2nd injection (morning); seine blue catfish (2 blue catfish for every 10 females) and hold them in hatchery. Day 3) Kill 2 blue males, remove testes and macerate to prepare sperm solution. Strip spawn eggs, fertilize, and hatch them in troughs.

Checking ovulating females: Slightly lift the suspended spawning bag above the water. Typically, ovulating fish has few eggs along the seam of the bag – mark the bags with ovulating fish with plastic clips.

Sedate the ovulating fish in the bag. Remove the fish from the bag and dip the fish in rinsing solution. Dry the fish with a towel and wrap the fish around the head and pectoral fins. Roll the fish on her back, holding the tail with the left hand and stroke the fish from the right hand towards the vent to see a flow of eggs from the vent. Strip the eggs in greased bucket or pans.

DO NOT HAVE CONTACT WITH WATER DURING ANY OF THESE PROCEDURES.

Quantify the stripped eggs, remove clumps, and wash away blood and body fluids with saline solution. Fertilize the eggs with blue catfish sperm solution (1 gram of testes for 250 ml of eggs). Pour series of greased pans with 150 to 200 ml of fertilized eggs.

Activate the egg and sperm solution by adding a cup of water to the egg and sperm mix and gentle stir it with a plastic spoon and set it for 1 minute. Rinse of the excess water slowly without disturbing the spawn and slowly add water on the side of the pan and fill it up to the maximum and allow it to settle for 10 minutes. Slowly flip the developing spawn in the pan and allow it to settle for 5 minutes. Transfer the pans with the spawn into a hardening tank that has water to a depth of 4 -6 inches and is also provided with continuous water and air. After 20 to 30 minutes, remove the pans with the freshly formed spawn and transfer it to hatching baskets.

Typically 2 spawns can be transferred to a basket suspended in hatching troughs. Continuous water and air will keep the spawn agitated; paddles can be operated in 2 hours or so to reduce the mechanical damage on fresh spawn. It takes 4 days for the fertilized eggs to hatch at 28° C.

Blue catfish male

Removing testes

Good quality testes

Rinsing testes in HBSS to clean

Crushing testes

Measuring sperm solution

Literature Cited

Bosworth, B.G., W.R. Wolters, J.L. Silva, R.S. Chamul, and S. Park. 2004. Comparison of production, meat yield, and meat quality traits of NWAC 103 line channel catfish (*Ictalurus punctatus*), Norris line channel catfish, and channel catfish female x blue catfish male (*I. furcatus*) F1 hybrids. North American Journal of Aquaculture 66:177-183.

Chatakondi, N.G., J. Benfer, L.S. Jackson, and D.R. Yant. 2000. Commercial evaluation of channel x blue hybrid catfish production and their performance in ponds. Presented at the 2000 Catfish Farmers of America Research Symposium at Albuqerque, NM.

Christensen, J.M. and T.R.Tiersch. 1996. Refrigerated storage of channel catfish sperm. Journal of the World Aquaculture Society 27:340-346.

Dunham, R.A. and B. Argue. 1998. Seinability of channel catfish, blue catfish, and F1, F2, F3 and backcross hybrids in earthen ponds. Progressive Fish-Culturist 60:214-220.

Dunham, R.A. and R.E. Brummett. 1999. Response of two generations of selection to increased body weight in channel catfish, *Ictalurus punctatus* compared to hybridization with blue catfish, *I. furcatus*, males. Journal of Applied Aquaculture.9:37-45

Dunham, R.A., R.O. Smitherman, M.J. Brooks, M. Benchakan, and J.A. Chappell. 1982. Paternal predominance in channel-blue hybrid catfish. Aquaculture 29:389-396.

Dunham, R.A., R.O. Smitherman, and C. Webber. 1983. Relative tolerance of channel x blue hybrid and channel catfish to low oxygen concentrations. Progressive Fish-Culturist 45:55-56.

Dunham, R.A., R.O. Smitherman, and R.K. Goodman. 1987. Comparison of mass selection, crossbreeding and hybridization for improving body weight in channel catfish. Progressive Fish-Culturist 49:293-296.

Dunham, R.A., R.E. Brummett, M.O. Ella, and R.O. Smitherman. 1990. Genotype-environment interactions for growth of blue, channel and hybrid catfish in ponds and cages at varying densities. Aquaculture, 85:143-151

Dunham, R.A., Z.J. Liu, and B. Argue. 1998. Effect of the absence or presence of channel catfish males on induced ovulation of channel catfish females for artificial fertilizations with blue catfish sperm. Progressive Fish-Culturist 60:297-300.

Dunham, R.A., D.M. Lambert, B.J. Argue, C. Ligeon, D.R. Yant, and Z. J. Liu. 2000. Comparison of manual stripping and pen spawning for production of channel catfish x blue catfish hybrids and aquarium spawning of channel catfish. North American Journal of Aquaculture 62:260-265.

Dupree, H. K., O. L. Green, and K.E. Sneed. 1969. Techniques for the hybridization of catfishes, Publ. 221, U.S. Fish Wildlife Service. Southeast Fish Cultural Laboratory, Marion, AL, 9p.

Giudice, J.J. 1966. Growth of a blue X channel catfish hybrid as compared to its parent species. Progressive Fish-Culturist 28:142-145.

Graham, K. 1999. A review of the biology and management of blue catfish. American Fisheries Society Symposium 24:37-49.

Li, M.H., B.B. Manning, E.H. Robinson, R.D. Yant, N.G. Chatakondi, B.G. Bosworth, and W.R. Wolters. 2004. Comparison of the channel catfish, *Ictalurus punctatus*, (NWAC 103 strain) and the channel x blue catfish, *I. punctatus* x *I. furcatus*, F1 hybrid for growth, feed efficiency, processing yield, and body composition. Journal of Applied Aquaculture 15(3/4):63-71.

Ligeon, C.M. 1993. The effect of feeding different levels of protein and supplemental liver on the spawning rate, fertilization rate, and egg hatchability of different genotypes of channel catfish and blue catfish. M.S. Thesis, Auburn University, AL.

Ramboux, A.C. 1990. Evaluation of four genetic groups of channel-blue catfish hybrids grown in earthen ponds. Ph. D. Dissertation, Auburn University, AL.

Tave, D.L. and R.O. Smitherman. 1982. Spawning success of reciprocal hybrid pairings between blue and channel catfishes with and without hormone injection. Progressive Fish-Culturist 44:73-74.

Wolters, R.W., D.J. Wise, and P.H. Klesius. 1996. Survival and antibody response of channel catfish, blue catfish and channel catfish female x blue catfish male hybrids after exposure to *Edwardsiella ictaluri*. Journal of Aquatic Animal Health 8:249-254.

Yant, R., R.O. Smitherman, and O.L. Green. 1975. Production of hybrid (blue X channel) catfish and channel catfish in ponds. Proceedings Annual Conference Southeast. Association of Game and Fish Commissioners 29:86-91.

Care of Hybrid Catfish Egg Masses

One aspect of hybrid production that requires strict attention to detail is treatment of eggs for bacteria and fungus. Catfish hatcheries typically have some sort of egg treatment protocol in place, but it is even more important to treat hybrid eggs aggressively. The main reason for a good egg treatment protocol is that even with the best technique it is difficult to produce hybrid egg masses with the levels of fertility commonly observed in pond-spawned channel catfish eggs. The unfertilized eggs provide excellent substrate for growth of bacteria and fungus which then spreads to the fertilized eggs and by the time fry are ready to hatch the majority of the fry are dead and hatch rate is greatly reduced.

Occasionally you will produce hybrid spawns with nearly 100% fertility and these egg masses hatch very well, but you may also get some with very low fertility (10-20%) that produce very few fry. Aggressive egg treatment may help in situations where you have 50–80% fertility. Without treatment, the hatch in a spawn with 50% fertility will be greatly reduced. Even with aggressive treatment, hatch results can be much lower than fertility rates.

Improving hybrid egg survival requires good husbandry practices and continuous attention to detail. Optimal health of the developing fry is best achieved with a good environment, reduced handling, and aggressive egg treatments. Unlike pond-spawned catfish, you can control the size and timing of the spawn. It is important not to make your egg masses to thick or treatments will not be as effective. It is also important to start treatments 8-12 hours post fertilization and possibly avoid treatment with any compound at 40-46 hours post fertilization window. Research suggests that this time frame may be a sensitive point for the developing embryos. The following recommendations are guidelines, and you find other treatment protocols to be effective. Regardless, hybrid catfish eggs require an aggressive treatment protocol for successful fry production.

Factors Leading to Dead Eggs and Disease

Strip-spawning can often result in egg masses with a large number of unfertilized or dead eggs. In the hatchery, over-handling, overcrowding, and adverse environmental factors, such high temperatures and poor water quality, also result in egg stress and death. Unfertilized and dead eggs are the primary target of disease causing pathogens and provide a starting point for diseases to spread. Even in the most sanitary of hatcheries, pathogens are present. Once a disease outbreak has begun, it can quickly get out of hand. Prevention should be the first goal of a good hatchery disease management plan.

Dead eggs

Dead eggs need to be managed to prevent massive disease outbreaks. Live eggs should appear transparent and progress from a pale yellow color to an orange–red color. Dead eggs are often difficult to observe during the first 1-2 days after spawning. By the third day, dead eggs typically appear opaque and colorless. Some dead eggs will also be enlarged. When dead eggs

are observed, they can be manually removed to prevent infection. When removing dead eggs, care must be taken not to damage good eggs.

Overcrowding

Many factors affect the maximum loading rate a hatchery can sustain. Generally, two large egg masses (approx. 1 kg (2 lb) each) or three small egg masses (approx. 0.5 kg (1 lb) each) can be incubated in a single hatching basket 20 cm (8 in) wide x 41 cm (16 in) long x 10 cm (4 in) deep). Egg masses that overlap substantially are subject to poor water circulation, reduced egg survival, and the direct transfer of diseases between egg masses.

Temperature

Temperature is an important environmental factor affecting fry development, hatch rates, and disease susceptibility. The optimal temperature range for incubating catfish eggs is between 26–28^{o}C (78-82^{o} F). At temperatures above and below this range, egg death and prevalence of disease increases, reducing hatch rates (Figure 1).

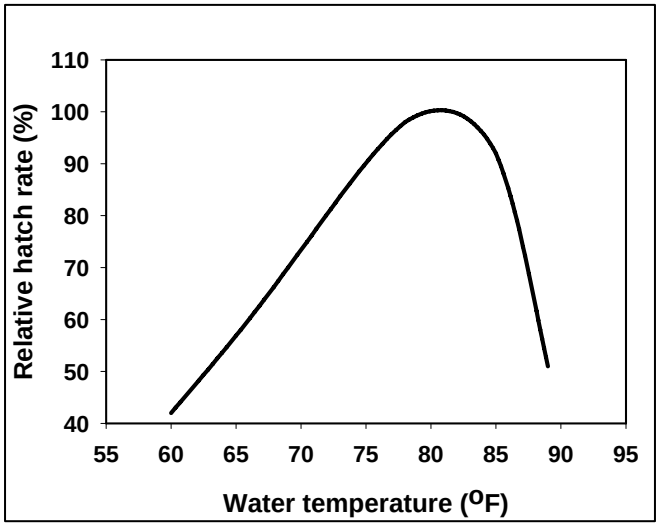


Figure 1. Effect of water temperature on catfish hatch rates.

The time it takes for catfish eggs to hatch also depends on water temperature. Catfish typically spawn in the spring, when water temperatures are between 21 – 29^{o} C (70-84^{o} F). At these temperatures, the time to hatch is between 5 and 10 days (Figure 2).

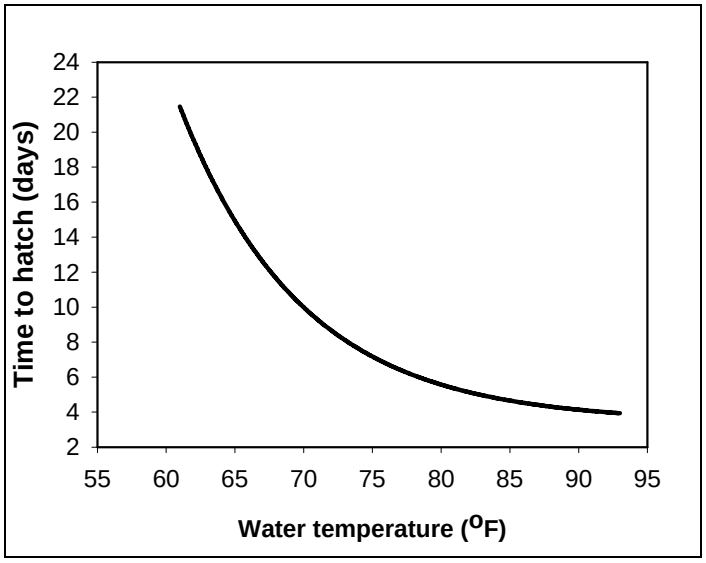


Figure 2. Effect of water temperature on time to hatch catfish eggs.

Water hardness

Water hardness plays an important role in catfish fry development. Low calcium levels in hatchery water can increase egg death and reduce hatch rates. Hatch rates from eggs incubated in water with less than 10 ppm calcium-hardness during the first 24 hours after spawning are reduced by as much as 70%. Low calcium-hardness during later stages of development can cause up to a 25% reduction in hatch rates. For this reason, it is important to maintain adequate water hardness in the hatchery. It is recommended that a minimum calcium-hardness of 20 ppm be maintained, especially during the first 24 hours after spawning.

Disease Causing Organisms

Bacterial and fungal infections are the primary threats when incubating catfish eggs. Generally, bacterial infections occur when hatchery water temperatures are above 28^{o} C (82^{o} F) and when egg masses are overcrowded. Bacterial egg rot appears as a milky-white patch in the egg mass. This patch of bacteria will contain dead and deteriorating eggs, and is often seen on the underside and in the middle of the egg mass. Anytime milky-white patches are observed on the egg mass, care should be taken in the removal of the bad spot and surrounding dead eggs.

Fungus is more prevalent at lower temperatures, usually 26^{o} C (78^{o} F) and below, and rapidly attacks infertile and dead eggs. Fungal infections are easy to spot, appearing as white or brown cotton-like growths made of many small filaments. If left untreated, these filaments can invade and kill adjacent healthy eggs, expanding to cover the entire egg mass and potentially every egg mass in the hatching trough. Mechanical removal of dead and infected eggs can be time consuming, but is beneficial. Chemical control of fungal infections is quite effective. Regular disinfection of eggs with chemical disinfectants is commonplace in most commercial catfish hatcheries.

Chemical Disinfection

The use of drugs and chemicals for disinfecting eggs in aquaculture facilities is regulated by various federal and state agencies. Treatments must be effective, safe, and cost efficient. The United States Food and Drug Administration (FDA) is responsible for ensuring the safety and effectiveness of aquaculture drugs, including those chemicals used to treat diseases of fish eggs. There are presently four options for the legal use of chemotherapeutants in the United States: (1) the chemical has been approved by the FDA; (2) the chemical is the subject of an Investigational New Animal Drug (INAD) exemption; (3) the chemical has been determined by the FDA to be of low regulatory priority; and (4) the chemical is not low regulatory priority, but regulatory action has been deferred pending the outcome of ongoing research. More information on obtaining and using drugs and chemicals for aquaculture purposes can be acquired through the FDA's Center for Veterinary Medicine (CVM; http://www.fda.gov/cvm/index/aquaculture/aqualibtoc.htm), which regulates the manufacturing, distribution, and use of animal drugs.

Several factors must be considered when developing a chemical treatment strategy for managing egg disease. Biological, environmental, and physical factors all play a role in the effectiveness of the treatment. Each hatchery is unique in the way it's built, the source and quality of water, the capacity of the facility, and the management. It is highly recommended that visits be made to existing hatcheries to discuss their disease management strategies. The following discussion of chemical treatment methods is meant to be a guideline for hatchery managers and is based on research, experience, and personal communications with hatchery managers and extension specialists.

Biological factors

The primary biological factor to consider is the age of the developing eggs. Many studies have been conducted to determine the effects of treating eggs at various stages of development. In general, the egg is a very tough protective environment for the developing fry. When using the correct concentration, length, and frequency of treatment, the developing fry is protected up to the time it hatches from the egg. As such, treatments should be continued until the "eyed" egg stage, the point in time when eye pigmentation (black eye spots) can be observed without magnification. Newly hatched fry are vulnerable to chemical disinfectants.

The best hybrid hatch rates have been achieved by treating three times daily with formalin; however, research has suggested that treatments should not be conducted 40-46 hours post-fertilization, since this stage of development may be chemically sensitive. If trying to salvage severely diseased egg masses with more frequent or prolonged treatments, an isolated incubation trough should be used.

Environmental factors

Temperature and water quality not only affect the development and survival of developing catfish fry, but they can have a significant impact on the effectiveness of the chemical. Toxicity of some chemical treatments is directly proportional to temperature; as temperature increases so

does the toxicity of the chemical. On the other hand, some chemicals work better at higher temperatures than at lower temperatures. Fortunately, for the few chemicals that are FDA-approved or of low-regulatory priority for use in disinfecting catfish eggs, temperature does not appear to have a significant impact on effectiveness or toxicity.

Organic load in the water system can also impact the effectiveness of chemical treatments. High concentrations of organics in hatchery water systems should be avoided. Organics provide a food source for pathogens and may increase diseases in the hatchery. High levels of organics can also reduce the effectiveness of chemical disinfectants such as formalin and hydrogen peroxide.

Physical factors

Water flow rates and volume are the two biggest factors affecting how chemical treatments will be administered and can greatly impact the effectiveness of the treatment. When determining chemical concentrations for egg disinfection, the volume of water being treated must be precisely known. If eggs are to be treated as a bath in the hatching troughs, the time to completely turnover the volume should be factored into the decision of how long to treat the eggs, as part of the total time exposed to the disinfectant solution.

Turning off the water for bath treatments can be a substantial risk, with millions of eggs being lost in the event water flow is not restored. As an alternative, a flush treatment can be conducted in which the chemical is added to the trough with continuous water flow. The time it takes for one complete water exchange will dictate whether chemical concentrations must be increased or decreased.

Most recommendations for disinfecting catfish eggs suggest that the eggs be exposed to the treatment for 15 minutes at a given concentration. During flush treatments, faster water turnover (higher flow rates) must be compensated for by increasing the chemical concentration. Slower turnover rates provide for longer contact time between the egg mass and the chemical solution and require a reduction in the concentration of chemical used.

There are many opinions as to how long and how often eggs should be treated. Treatment durations that are too short and infrequent will not sufficiently kill the disease-causing pathogen, but treatment durations that are too long or too frequent may be toxic to the developing fry. In both cases, fry survival will be unacceptably low. A good hatchery manager will use the guidelines as a starting point and adjust treatment methods accordingly.

Disinfectants

Formalin. Formalin is an FDA-approved aquaculture drug for the control of fungi on all fish eggs. Formalin products for egg disinfection can currently be purchased under the trade names Formalin-F (Natchez Animal Supply Co.), Paracide-F (Argent Laboratories, Inc.), and Parasite-S (Western Chemical, Inc.). The maximum approved treatment concentration for use in disinfecting catfish eggs is 2000 ppm for 15 minutes as a flush treatment. Under typical hatchery

conditions with an average of one complete water exchange every 45-60 minutes, 2000 ppm can be toxic to channel catfish eggs.

In most hatcheries, fungus can be controlled by treating with 100 ppm formalin for 15 minutes as a bath treatment. Turn the water off during treatment, but leave the paddles turning or air flowing from airstones. Flush completely with fresh water when treatment time has elapsed. As a flush treatment, concentrations between 100 and 400 ppm formalin have been used successfully at temperatures ranging from 24-30°C (75-86°F). Hatch rates tend to improve when formalin treatments are administered twice daily. Recommended formalin treatments are presented in Table 1.

Hydrogen peroxide. Hydrogen peroxide is currently an aquaculture drug of low regulatory priority according to the FDA. It is expected that hydrogen peroxide will eventually be approved by the FDA as a new animal drug, and that the label will include the treatment of catfish eggs. As a drug of low regulatory priority, permitted use of hydrogen peroxide includes the control of fungi on all life stages of fish, including eggs, at concentrations of 250-500 ppm on an active ingredient basis (100% hydrogen peroxide). Hydrogen peroxide is extremely caustic in its concentrated form and can be purchased as 3, 35, and 50% solutions. The most practical concentration for use as a chemical disinfectant is the 35% solution.

Table 1. Recommended volumes of formalin and hydrogen peroxide for use as flush treatments to disinfect hybrid catfish eggs in a hatching trough containing 100 gallons of water.

	Milliliters (fluid ounces)	
Water flow (GPM)	Formalin (37% formaldehyde solution)	Hydrogen peroxide (35% solution)
1.0	10 (0.3)	40 (1.4)
2.0	30 (1.0)	75 (2.5)
3.0	50 (1.7)	110 (3.7)
4.0	70 (2.4)	150 (5.1)
5.0	90 (3.0)	190 (6.4)
6.0	110 (3.7)	225 (7.6)

* Chemical volumes are provided as starting points and may require adjustment for unique hatchery conditions.
** Recommended treatment frequency is 3 times per day for formalin and 1 time per day for hydrogen peroxide.
*** *DO NOT treat eggs that are hatching.*

The effectiveness of hydrogen peroxide appears to be impacted by temperature, and may be the result of increased toxicity at higher temperatures. When hatchery water temperature is 26°C (78°F), a daily, 15-minute bath in a solution of 250 ppm active hydrogen peroxide (715 ppm of 35% hydrogen peroxide) is as effective as formalin at disinfecting eggs and improving hatch rates. It is important to note, however, that twice as much hydrogen peroxide at this temperature is toxic to the eggs. At cooler temperatures, toxicity is reduced and higher concentrations of hydrogen peroxide are more effective. Recommended hydrogen peroxide treatments are presented in Table 1.

Povidone iodine. Povidone iodine is also an aquaculture drug of low regulatory priority according to the FDA. Permitted use of povidone iodine compounds includes the disinfection of catfish eggs in a solution of 100 ppm for 10 minutes. Daily iodine treatments are not as effective as daily formalin treatments for controlling fungal infections. Povidone iodine is, however, a very good preliminary disinfectant when transferring eggs from the pond to the hatchery. A 10 minute bath in a 100 ppm iodine solution prior to adding new egg masses to communal incubating troughs can substantially reduce the transfer of pathogens from the pond to the hatchery and may improve hatch rates by as much as 10% when used in coordination with a daily disinfectant scheme of either formalin or hydrogen peroxide.

Copper sulfate. Copper sulfate currently falls under the FDA label of investigational new animal drug (INAD), and regulatory action has been deferred pending the outcome of ongoing research. Compounds in the INAD category are used under an investigational new animal drug exemption administered by the FDA/CVM to allow for the purchase, shipment, and use of unapproved new animal drugs for collection of effectiveness and safety data that will support a decision for drug approval. Data in support of copper sulfate as an egg disinfectant is currently being collected for use in the drug approval process. Preliminary data suggests that copper sulfate is a very effective disinfectant for controlling fungal infections of catfish eggs.

As once or twice daily 15-minute bath treatments, copper sulfate concentrations of 2.5-10 ppm appear effective in reducing disease and improving hatch rates. Higher levels of copper sulfate have been found to reduce hatch rate, suggesting a toxic effect. When adding copper sulfate to the hatching trough, crystalline copper sulfate should be mixed in a 5-gallon bucket of hatchery water and added as a solution. Copper sulfate is not recommended for use in aluminum troughs since it reacts with the aluminum and causes the trough surface to become pitted.

Summary

General recommendations

Hybrid catfish eggs require greater attention to detail and aggressive treatment for bacteria and fungus. While it's clear that many factors can be attributed to poor hatch rates, knowing the optimal conditions for handling and hatching catfish eggs and following good hatchery practices will improve fry survival. Some recommendations include:

1. Avoid unnecessary handling of eggs.
2. Disinfect egg masses with povidone iodine prior to placement in a communal incubation trough.
3. Maintain hatchery water temperatures between 26–28^{o} C (78-82^{o} F) for optimal hatching success.
4. Avoid overcrowding of egg masses in troughs.
5. Maintain adequate water hardness in the hatchery to improve fry survival.
6. Be familiar with the laws regulating chemical use for disinfecting catfish eggs.
7. Treat eggs aggressively with an approved chemical disinfectant to manage diseases and improve hatch rates.

Timing of Treatments

1. Start treatments: 8-12 hours post fertilization.
2. Do not treat with any compound in the 40- to 46-hour post fertilization window. Applications of formalin during this period have been linked to egg loss.
3. Treat egg masses 3 times per day:
    - Early morning (6-7 a.m.)
    - Noon
    - Evening (6-7 p.m.)
4. Stop treatments when eggs begin hatching. Dip those masses that are not yet hatched if the delay is expected to be several hours.

Treatment Regimes

1. Static treatment: 15 minute bath.
    - Option 1: Three treatments per day with 100 ppm of formalin (37% formaldehyde).
    - Option 2: Three treatments per day with 100 ppm povidone iodine (1%).
    - Option 3: Three treatments per day with 2.5 ppm copper sulfate.
2. Flowing water treatment: In 100-gallon troughs with 3 gallons per minute of flow.
    - Option 1: Three treatments per day with 50 ml of formalin (37% formaldehyde).
    - Option 2: Three treatments per day with 50 ml of povidone iodine (1%).
    - Option 3: Two treatments per day with 50 ml of povidone iodine (1%) and one treatment per day with 10 grams of copper sulfate crystals. (Dissolve copper sulfate in 5 gallons of water and pour across trough.)

Additional Resources

Hargreaves, J.A. and C.S. Tucker. 1999. Design and Construction of Degassing Units for Catfish Hatcheries. SRAC Publication No. 191. Southern Regional Aquaculture Center.

Small, B.C. 2004. Accounting for water temperature during hydrogen peroxide treatment of channel catfish eggs. North American Journal of Aquaculture 66:162-164.

Small, B.C., W.R. Wolters, and T.D. Bates. 2004. Identification of a calcium-critical period during channel catfish embryo development. Journal of the World Aquaculture Society 34:313-317

Small, B.C. and W.R. Wolters. 2003. Hydrogen peroxide treatment during egg incubation improves channel catfish hatching success. North American Journal of Aquaculture 65:314-317.

Small, B.C. and T.D. Bates. 2001. Effect of low-temperature incubation of channel catfish, *Ictalurus punctatus*, eggs on development, survival and growth. Journal of the World Aquaculture Society 32:49-54.

Steeby, J.A. and J.L. Avery. 2005. Channel Catfish Broodfish Selection and Hatchery Management. SRAC Publication No. 1803. Southern Regional Aquaculture Center.

Tucker, C.S. and J.A. Steeby. 1993. A practical calcium hardness criterion for channel catfish hatchery water supplies. Journal of the World Aquaculture Society 24:396-401.

Tucker, C.S. and E.H. Robinson. 1990. Channel Catfish Farming Book. Van Nordstrand Reinhold: New York, New York.

Tucker, C.S. and J.A. Hargreaves. 2004. Biology and Culture of Channel Catfish. Elsevier, Amsterdam, The Netherlands.

Tucker, C.S. 1991. Water Quantity and Quality Requirements for Channel Catfish Hatcheries. SRAC Publication No. 461. Southern Regional Aquaculture Center.

Walser, C.A. and R.P. Phelps. 1993. The use of formalin and iodine to control *Spaprolegnia* infections on channel catfish, *Ictalurus punctatus*, eggs. Journal of Applied Aquaculture 3:269-278.

Wedemeyer, G.A. 2001. Fish Hatchery Management, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Appendix

LIST OF SUPPLIES AND VENDORS:

Carp Pituitary Extract	**Stoller Fisheries** P.O. Box B (Mailing address) 1301 18th Street (Physical address) Spirit Lake, Iowa 51360 800-831-5174, 712-336-1750 fax 712-336-4681 e-mail: stollerfisheries@mchsi.com website: http://www.sfishinc.com
Catfish Pituitary Extract	**Hybrid Catfish Company** Roger Yant 1223 Montgomery Drive, Inverness, MS 38753 662-207-0461
LHRHa (cat # 13440)	**Syndel International Inc.** 9211 Shaughnessy Street Vancouver, British Columbia V6P 6R5, Canada 800-831-5174, 712-336-1750 fax 712-336-4681,e-mail: info@syndel.com website: http://www.syndel.com
LHRHa Implants	**Eagle Aquaulture, Inc**., Suite 5 – PMB 157 Auburn, AL 36830. 334-737-3100 Sam.lawrence@aetostech.com
Spawning Bags	**WebSupply LLC**, P. O. Box 1370 Fairfield, CT 06825. Tel : 203-400-0939 Chester@Websupply.US
Lab Supplies (Syringes/needles Glassware, Scalpels, Volumetric Flasks, Tubes)	**A Daigger & Co. Inc.** 620 Lakeview Parkway Vernon Hills, IL 60061 800-621-7193, fax 800-320-7200 website: http://www.daigger.com
Chemical Supplies (General Chemicals, Pre-made PBS)	**Fisher Scientific** 800-766-7000 website: http://www.fishersci.com **Sigma Aldrich** 800-325-3010 website: http://www.sigmaaldrich.com

Tranquilizers and some of the items listed above (syringes, needles etc.) can be purchased from an aquaculture supply store. Spawning buckets, testes strainers, no-stick oil, towels etc. can be purchased from many retailers. Mention of any trade name or vendor does not imply endorsement by the USDA or Mississippi State University.

RECIPES:

Hanks Buffered Salt Solution (HBSS) for English units (gallons)

Ingredient	1 gal	5 gal	25 gal
Sodium chloride (NaCl) You can use uniodized salt from the grocery store, make sure you keep it dry.	30.05 g	150.25 g	751.25 g
D-Glucose (dextrose) Buy anhydrous (no water), Fisher Scientific sells a reagent grade (Catalog # S734181) 1 kg (1000 g or ~ 2.25 lbs) for ~ $12.15	3.79 g	18.95 g	94.75 g
Potassium chloride (KCl) Fisher Scientific sells a reagent grade (Catalog # S773751), 500 g for ~ $7.85	1.52 g	7.60 g	38.0 g
Sodium bicarbonate ($NaHCO_3$) Fisher Scientific sells a reagent grade (Catalog # S78284), 500 g for ~ $5.65 You can also use baking soda.	1.32 g	6.6 g	33.0 g
Potassium phosphate (anhydrous) (KH_2PO_4) Fisher Scientific sells a reagent grade (Catalog # S801462), 100 g for ~ $8.50	0.23 g	1.15 g	5.75 g
Sodium phosphate (dibasic anhydrous) (Na_2HPO_4) Fisher Scientific sells a reagent grade (Catalog # S75218), 100 g for ~ $9.45	0.19 g	0.95 g	4.75 g

To mix HBSS for testes preparation, use distilled or deionized water. A good alternative is bottled water that is commonly sold in stores, most brands of bottled water go through a series of filters and ozonation so they are basically distilled. Any brand bottled water should work. Using sterile water for testes preparation is not really necessary, but if you want you can sterilize it by boiling in a glass bottle with the lid loosened and place in a pot of boiling water. Be sure to cool it before using.

To mix HBSS for egg rinsing, carefully measure out the volume by adding a gallon or liter at a time to a large plastic garbage can and then mark the volume. After that, just fill the can to that level and add the chemicals for the known volume. Use well water for egg rinse HBSS. Unless the ionic concentration of your well water is really strange it should be find for the egg rinse HBSS. Be sure HBSS is mixed well, and is has adequate oxygen, and don't use a chlorinated water source.

RECIPES:

Hanks Buffered Salt Solution (HBSS) for metric units (liters)

Ingredient	1 liter	10 liter	100 liter
Sodium chloride (NaCl) You can use uniodized salt from the grocery store, make sure you keep it dry.	7.94 g	79.4 g	794 g
D-Glucose (dextrose) Buy anhydrous (no water), Fisher Scientific sells a reagent grade (Catalog # S734181) 1 kg (1000 g or ~ 2.25 lbs) for ~ $12.15	1.0 g	10.0 g	100.0 g
Potassium chloride (KCl) Fisher Scientific sells a reagent grade (Catalog # S773751), 500 g for ~ $7.85	0.4 g	4.0 g	40.0 g
Sodium bicarbonate ($NaHCO_3$) Fisher Scientific sells a reagent grade (Catalog # S78284), 500 g for ~ $5.65 You can also use baking soda = sodium bicarbonate.	0.35 g	3.5 g	35.0 g
Potassium phosphate (anhydrous) (KH_2PO_4) Fisher Scientific sells a reagent grade (Catalog # S801462), 100 g for ~ $8.50	0.06 g	0.6 g	6.0 g
Sodium phosphate (dibasic anhydrous) (Na_2HPO_4) Fisher Scientific sells a reagent grade (Catalog # S75218), 100 g for ~ $9.45	0.05 g	0.5 g	5.0 g

To mix HBSS for testes preparation, use distilled or deionized water. A good alternative is bottled water that is commonly sold in stores, most brands of bottled water go through a series of filters and ozonation so they are basically distilled. Any brand bottled water should work. If you use water that comes in a 1 liter or 500 ml bottle it is already measured out. 1 liter = 1000 ml (two 500 ml bottles = 1 liter). Using sterile water for testes preparation is not really necessary, but if you want you can sterilize it by boiling in a glass bottle with the lid loosened and place in a pot of boiling water. Be sure to cool it before using.

To mix HBSS for egg rinsing, carefully measure out the volume by adding a gallon or liter at a time to a large plastic garbage can and then mark the volume. After that, just fill the can to that level and add the chemicals for the known volume. Use well water for egg rinse HBSS. Unless the ionic concentration of your well water is really strange it should be find for the egg rinse HBSS. Be sure HBSS is mixed well, and is has adequate oxygen, and don't use a chlorinated water source.

RECIPES:

PHOSPHATE BUFFERED SALINE (PBS)

PBS is used to mix the powdered carp pituitary extract and LHRHa for injection. Any physiological saline (ionic makeup similar to body fluids) would work, but PBS is commonly used and can be purchased already made from many vendors. Although a recipe for PBS is included, it is recommended that pre-made PBSbe purchased. It will save you time and you won't have to worry if you mixed it correctly.

Use distilled or bottled water to make PBS or dilute purchased PBS if necessary. Since you are going to inject this in the fish you would probably have fewer problems with infections if you sterilize the water as suggested earlier.

Fisher Scientific sells a 1X (1X means it is ready to use as is) PBS (cat # BP2438-4) which is 4 liters for $80.75. This is the smallest amount of 1X PBS that they sell, and is enough to inject about 20,000 lbs of fish.

Fisher Scientific also sells a 10X (10X means it needs to be diluted 1 to 10 prior to use, so you add 1 part 10X PBS to 9 parts water to give PBS ready to use) (cat # BP399-500) for 500 ml (500 cc) for $22.12. This will make 5000 ml (5000 cc) of ready to use PBS which is enough to inject about 25,000 lbs of fish. Therefore, it is suggested to buy the 10X and dilute it.

Fisher Scientific also sells a powder that you add 10 liters of water to make 10 L of ready to use PBS for $32.04 (catalog # BP661-10).

Sigma Aldrich sells a similar liquid 500 ml, 10X PBS for $22.70 (catalog # D1283).
Sigma Aldrich also sells a powder, you add water, to make 10 L of PBS for $9.90 Catalog # D5773).

An internet search will turn up lots of other vendors of PBS.

If you want to make your own PBS you can use the following recipe.

Phosphate Buffered Saline*	1 liter	1 gallon
Salt (NaCl)	8 g	30.3 g
Potassium Chloride (KCl)	0.2 g	0.76 g
Sodium phosphate (Na_2HPO_4)	1.44 g	5.45 g
Potassium phosphate (KH_2PO_4)	0.24 g	0.91
Adjust pH to 7.4 with dilute hydrochloric acid		
Autoclave to sterilize		

* Same chemicals are listed for HBSS so you can use vendors listed for HBSS.

INVESTIGATIONAL NEW ANIMAL DRUG (INAD) PERMIT

- An INAD permit is required for use of LHRH or Carp Pituitary (CCP) if you intend to sell fish for human consumption.
- Issued by U.S. Fish and Wildlife Service. Intent is to collect data to support FDA approval of drug.
- Cost is $400.00 per INAD.
- Maximum dose allowed for LHRH is 100 ug/kg (45.5 ug/lb.) and 10 ug/kg (4.5 ug/lb) for CCP.
- Required forms (copies on following pages)
    - Study design worksheet
    - Receipt of drug report form
    - Drug inventory form
    - Results report form
- There is a 30 day withholding period before you can sell broodfish that have been injected with either CCP or LHRH.
- Monitor required – person reviews forms and sends to U.S.F.W.S.
- Results include # of females injected, # of females that release eggs, percent eyed eggs, and percent hatch.
- Requires some paperwork but most of the data collected will be useful to you.
- INAD is not designed to prevent you from using the hormones but is intended to help collect data so that these compounds can be approved for commercial use.

Contact person for INAD information:

Bonnie Johnson*
US Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Rd
Bozeman, MT 59715
406-587-9265 ext. 105
406-582-0242 fax
http://fisheries.fws.gov/aadap/

* Bonnie is very helpful and the turnaround time on getting an INAD is quick, about 2 days.

SRAC Publications Links: (https://srac.tamu.edu/index.cfm/event/viewAllSheets/)

0190 - Production of Hybrid Catfish
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/31/

0421 - Introduction to Hormone-Induced Spawning of Fish
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/84/

0422 - Capturing, Handling, Transporting, Injecting and Holding Brood Fish for Induced Spawning
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/85/

0424 - Hormonal Control of Reproduction in Fish for Induced Spawning
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/87/

0425 - Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/88/

0426 - Techniques for Taking and Fertilizing the Spawn of Fish
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/89/

1802 - Channel Catfish Broodfish Management.
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/152/

1803 - Channel Catfish Broodfish and Hatchery Management
https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/153/

SRAC 17th Annual Progress Report 'Improving Reproductive Efficiency to Produce Channel x Blue Hybrid Catfish Fry' pp 61-76
http://srac.msstate.edu//apr17.pdf

Publications, Dissertations, Thesis, and Presentations generated By 'Improving Reproductive Efficiency to Produce Channel x Blue Hybrid Catfish Fry' SRAC Project
http://srac.msstate.edu//bluehybr.htm

NWAC Workshop: Production of Channel x Blue Catfish Hybrid Fry


Thad Cochran National Warmwater Aquaculture Center,
Stoneville, MS
April 6 and 7, 2011

Lecture

Day 1

- Basics of hormone induced spawning.
- Facility/broodfish requirements.
- Female selection.
- Pituitary induced spawning.
- Stripping females.
- Male selection, testes preparation.
- Scheduling for pituitary induced spawning.
- LHRHa induced spawning.

Day 2

- Jar Hatching
- Egg treatments

Hands-On

Day 1

- Mixing HBSS - CGRU Hatchery
- Mixing pituitary extract.
- Selecting females/injecting.
- Selecting males/preparing testes.
- LHRHa bags technique.

Day 2

- Checking sperm motility - NWAC
- Stripping eggs/fertilization - CGRU Hatchery
- Jar hatching.
- Egg treatment.

Hormone Induced Spawning

- Females injected with hormones to induce ovulation (release of eggs).
- Two main products used to spawn catfish:
  - **Pituitary Extract: carp or catfish.**
  - Part of brain that sends signals leading to ovulation.
  - Crude preparation, mixture of hormones.
  - Recommended for first time users.
  - More consistent timing, may work better early season.
  - **LHRHa (Luteinizing Hormone Releasing Hormone)**
  - Synthetic product.
  - Different approach and timing.
  - Better results???

Induced Spawning of Catfish

- 3 main methods:
  1) pair-spawning.
  2) group-spawning.
  3) bag-spawning (Nagaraj).
- Pair-spawning:
  - channel male and female put in a tank.
  - females observed for release of eggs then stripped.
- Group-spawning:
  - group of females in a large tank.
  - checked for release of eggs periodically.
  - females releasing eggs are stripped.
- Tank water temperatures should be 78-82 F, good water quality, D.O., minimal disturbance.

Important Factors

- Good quality broodfish.
- Proper hormone dosage/timing.
- Proper preparation of testes/sperm.
- Good prediction of ovulation.
- Good egg fertilization technique.
- Aggressive egg treatment.

Good Females Critical!

- Can deal with all other issues, poor females can't be fixed.
- Best to have mix of ages/sizes/strains.
- Want fish 'ready' at different times.
- Good management/forage over-winter.
- Stocking densities? 1500 to 2500 lbs acre.
- Don't need many (if any) males with them.
- When do you start?
  - When the fish are ready.
- When do you quit?
  - You will know.

Broodfish Requirements- Females

- How many females do you need?
  - Probably inject about 1/3 of what you have.
    - Some never ready, some you miss.
    - Injecting poor females is a waste.
    - 100 females/day for 30 days = 9,000 females
- How many ponds for females?
  - Seining same pond repeatedly is stressful for fish.
  - Big producers 6 to 8 ponds of brooders.
  - Small ponds better.
- When to start?
  - When females are ready.
- When to quit?
  - Fish will let you know.

Broodfish Requirements- Males

- How many males do you need?
  - Probably use about 200 to 400 good males, 1500 males total.
    - But need to be old to be mature.
    - Availability issues.
    - Probably 1 pond is plenty, may want 2 in case of disaster.
- Germplasm release?
  - USDA could supply blue catfish.
  - Mechanism?

Facility Requirements

- Increased water requirements
  - Need to hold females for several days.
  - Minimum of 4 vats.
  - Various types of vats.
- Poorer fertility and hatch.
  - Hatching space ~ double for same fry output.
- Less efficient but more consistent.
  - not affected by changes in weather.
- Increased frequency and importance of egg treatment.
- Increased fry production costs.

Overview- Pituitary Group Spawning of Catfish

- A 3 day process.
- Day 1
  - Seine/select channel females, transport to tanks.
  - Weigh/inject females with 1st hormone dose (afternoon).
- Day 2
  - Weigh/inject females with 2nd hormone dose. If splitting into two groups inject 1 group ~ 8 AM and other group ~ 10 AM.
- Day 3
  - Kill blue males, remove testes, prepare sperm.
  - Check for ovulating females, sedate and strip ovulated females, fertilize (blue catfish sperm).
- 80-100 females/day with a 6-7 person crew.

Pituitary Injection Times/Schedule

- Pituitary easy to establish schedule/timing.
- 1st injection 2mg/kg, timing not critical.
  - some give in morning, some in afternoon.
- 2nd injection 8 mg/kg, timing critical.
  - Spawning 24 to 30 hours after 2nd injection.
- 2nd Injection: 50 fish at 8 AM, another 50 at 10 AM.
  - Will spawn around 1 PM and 3 PM next day.
  - Depends on water temperature, time of year etc.
  - Fairly consistent timing once established.

100 fish/day - 6 days/week

- **Saturday**
  - Bring in 300 good females
  - Give 1st injection group 1
- **Sunday**
  - Give 2nd injection group 1
  - Give 1st injection group 2
- **Monday**
  - Spawn group 1
  - Give 2nd injection group 2
  - Give 1st injection group 3
- **Tuesday**
  - Spawn group 2
  - Give 2nd injection group 3
  - Bring in 300 females
  - Give 1st injection group 4
- **Wednesday**
  - Give 2nd injection group 4
  - Give 1st injection group 5
  - Spawn group 3
- **Thursday**
  - Give 2nd injection group 5
  - Give 1st injection group 6
  - Spawn group 4
- **Friday**
  - Give 2nd injection group 6
  - Spawn group 5
- **Saturday**
  - Spawn group 6
  - New fish, start cycle again.
- **Prep males every morning!**

Mixing and dosage: Pituitary

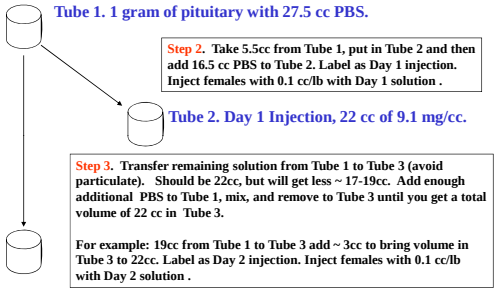
- Pituitary vendors
  - Carp: Stoller Fisheries.
  - Catfish: Hybrid Catfish Company
- Dry powder in 1 gram = (1000 mg) vials.
- Two injections – priming dose 2mg/kg, followed by resolving dose 8mg/kg.
- Store powder in dark at room temperature.
- Mix 1 gram CCP in 27.5cc (1cc = 1ml) of sterile saline solution (PBS). Shake and let sit in refrigerator for 1-2 hours.
- Take 5.5cc of this initial solution (avoid particulate at bottom of tube), add to another tube with 16.5 cc of PBS (22cc of 9.1 mg CCP/cc), use for 1st injection.
- Take remaining solution from initial tube, screen out particulate and bring volume up to 22cc.
- You will only get 18-19 cc. Add enough PBS to bring to 22cc, use for 2nd injection.

Pituitary - continued

- Label tube (Day 1 or Day 2), store in refrigerator. If excess use the next day.
- Use 3 to 10 cc syringe with 18 ga. needle, inject females behind pelvic fin.
- Mixed as described, 1 gram CCP give 22cc of Day 1 and 2 solutions, enough for ~ 220 lbs of females.
- Mixed as described, both 1st and 2nd injection are given at a 0.1cc/lb of body weight.
  - 5 lb female = 0.5cc Day 1 and 0.5cc Day 2 solution.
  - 10 lb female = 1.0cc of Day 1 and 1.0cc Day 2 solution.
- SRAC publication # 425 has equations for calculating dosage and volume if you want to use different injection volumes.

Mixing CCP

Step 1. Add 1 g CCP and 27.5 cc of PBS to Tube 1. Mix and store in refrigerator for 1-2 hours. Concentration in Tube 1 is 36.4 mg CCP/cc.



Tube 3. Day 2 Injection, 22 cc of 36.4 mg/cc

CCP Dosage Table

1 gram of CCP injects ~ 220 lbs of females. Mixed as suggested, the same volume will be injected at both injections. 1st injection is 0.91 mg/lb (2 mg/kg), 2nd injection is 3.64 mg/lb (8mg/kg).

Female weight (lbs)	Injection volume (cc)	Female weight (lbs)	Injection volume (cc)
3	0.30	8.0	0.80
3.5	0.35	8.5	0.85
4.0	0.40	9.0	0.90
4.5	0.45	9.5	0.95
5.0	0.50	10.0	1.00
5.5	0.55	10.5	1.05
6.0	0.60	11.0	1.10
6.5	0.65	11.5	1.15
7.0	0.70	12.0	1.20
7.5	0.75	12.5	1.25

Broodfish selection – channel catfish females

- Channel females ≥ 3 years or older.
- Broodfish management similar to pond-spawning.
- Higher stocking densities, fewer males than pond-spawn.
- No feed for ~ 3 days before selecting, full belly main method used to select female.
- Select females with:
  - full, soft, swollen belly.
  - swollen, red vent.
  - gray color under jaw.
- Fish releasing eggs at seining (ovulated in pond) not good.
- Don't inject poor quality females.
- Minimize stress (low D.O., hot temperatures etc.).
- Transport to holding tanks on oxygen.

Channel catfish female, swollen belly, good candidate for injection.

Injecting female behind pelvic fin.

Close-up of injecting female.

Testes Preparation

- Blue males should be ≥ 4 years old.
- Difficult to predict testes development.
  - Development of papilla.
  - Darker coloration on belly.
- Well developed testes: large, white, feathery.
- About 1 male per 10 females injected.
  - Depends on size and development of testes.
  - 1 gram of testes per 250 to 500 ml of eggs.

Preparing Testes of Blue Males

- Kill male by blow to head.
- Dry male well.
- Split open belly cavity, remove testes.
- Rinse testes in Hanks.
- Crush testes into Hanks to release sperm.
  - Various methods – window screen, Ziploc, blenders.
  - Don't damage sperm.
- Suspend in 'appropriate' volume of Hanks.
  - 40 females, 500 mls/female = 20,000 mls (20 liters) of eggs.
  - 250 mls (cc's) eggs per pan = 80 pans.
  - 10 cc's sperm per pan = 800 cc's total sperm solution.
- Store on ice, can be used for 48 hours if kept cold.
- Keep sperm "dry" until use.

Testes Preparation

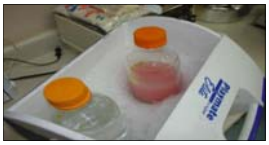
- Sperm activated (becomes motile) when exposed to fresh water.
- Motility only lasts ~ 60 seconds.
- Keep in HBSS before use, similar salt content to body fluids.
- DON'T activate sperm prior to mixing with eggs.
- Check sperm motility with a basic microscope.
  - Add drop of water to a microscope slide.
  - Add small drop of sperm, put cover slip over the drop.
  - Look for movement, should be motile 30 – 60 seconds.
  - Microscope from school or veterinarian.

Blue catfish male with abdomen opened for testes removal.

Good quality testes, feathery projections white in color.

Testes ready to be crushed and sieved through screen.

Crushing testes and sieving in HBSS to make sperm solution.



Sperm in HBSS, stored on ice.

Checking sperm motility, sperm is activated by water, when you add water you should see motile sperm.

Checking Females for Ovulation

- Look for egg release ~ 24 to 30 hours after 2nd injection at water temperature ~ 78-82 F.
- Time to ovulation varies:
  - longer if water is colder or early in spawning season.
- Put piece of 3-4" PVC in tank with fish. Released eggs will stick to the pipe.
- To check for ovulation, gently crowd fish and catch with a dip-net.
- Roll fish on her back, apply pressure with thumb and forefinger just ahead of the vent.
- If eggs flow 'fairly' freely, sedate fish, strip eggs.
- If eggs don't flow freely, check fish again 2-3 hours later.

Pipe placed in tank with injected females, check pipe for eggs as an indicator that females are starting to ovulate and release eggs.

Checking a female for egg flow to determine if she is ready to strip.

Stripping Channel Catfish Females

- Sedate ovulated fish with MS-222 (100 ppm).
- Rinse with hatchery water, then dry with a towel.
- Right handed: grasp just forward of tail with left hand, lay head of fish across right forearm, belly down, reach underneath fish with right hand.
- Place thumb and forefinger on opposite sides of belly, just ahead of pelvic fin, apply pressure moving hand back toward the vent. Eggs should flow out 'fairly' freely.
- Continue moving up belly as eggs empty from ovary.
- Collect eggs in a container with HBSS, previously sprayed with non-stick cooking spray.

Coat egg bucket with no-stick cooking spray, then add ~ 1 inch HBSS.

Female being stripped, eggs collected in HBSS in bucket.

Female catfish being dried off prior to egg collection.

Fertilization

- Good eggs flow smoothly, greenish-yellow, free of blood clots and clumps.
- Rinse eggs with HBSS, strain out large clumps.
- Pour into volumetric pitcher.
- Pour off HBSS, add ~ 200 to 250 ml per pie pan, add sperm solution.
- Stir eggs/sperm, add hatchery water (~ 3 to 1 water to eggs) to activate sperm.
- After ~ 5 minutes move eggs to trough, allow water to flow across.
- After egg mass sticks (10-30 minutes) move to hatching troughs.
- Some eggs don't stick, usually not good quality, but may get some hatch.

INAD (Investigational New Animal Drug)

- **INAD required for use of LHRH or Pituitary. Cost is $400.00 per INAD.**
- **Issued by U.S. Fish and Wildlife Service.**
- **Maximum total dose is 45.5 ug/lb (100 ug/kg) for LHRH and 4.5 mg/lb (10 mg/kg) for CCP.**
- **Required forms (included in Appendix)**
  - **Study design worksheet.**
  - **Receipt of drug report form.**
  - **Drug inventory form**
  - **Results report form**
- **Monitor required – person that review forms and sends to U.S. F&W Service.**
- **Results include # of females injected, # of females that release eggs, % eyed eggs, and % hatch.**
- **Some paperwork but information is useful.**
- **Contact for INAD information is:**

Bonnie Johnson
US Fish & Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Rd
Bozeman, MT 59715
406-587-9265 ext. 105
406-582-0242 fax
http://fisheries.fws.gov/aadap/

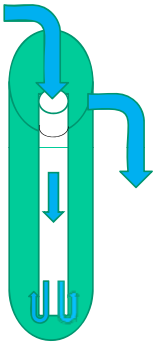
Jar Hatching

- 10” diameter tube, 6 ft long with flat end cap.
- Gravity flow.
- 250 k to 350 k eggs per jar.
- ~ 8 gallons/minute

Jar Hatching

- Advantages
  - Improved hatch
  - Less treatment
  - Less labor
  - Small footprint
  - Can salvage something from ‘bad’ eggs
- Disadvantages
  - Troubles with bubbles, inconsistent flow.
  - Potential for catastrophic failure!
  - Use of oxygen to minimize catastrophe.

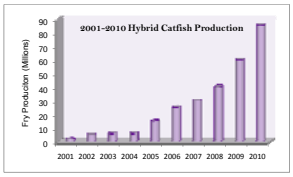
Jar Hatching



LHRHa induced ovulation of catfish in bags for hybrid catfish production.

Nagaraj Chatakondi
USDA ARS Catfish Genetics Research Unit Stoneville, MS

Hybrid Catfish
Channel catfish ♀ X Blue catfish ♂

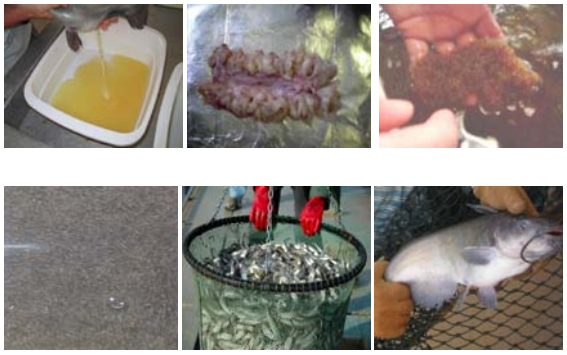


Advantages :
Increased growth rate
Lower feed conversion
Better Survival
Higher harvestability
Improved disease resistance
Better tolerance of low dissolved oxygen
Improved processing yield

Hybrid Catfish

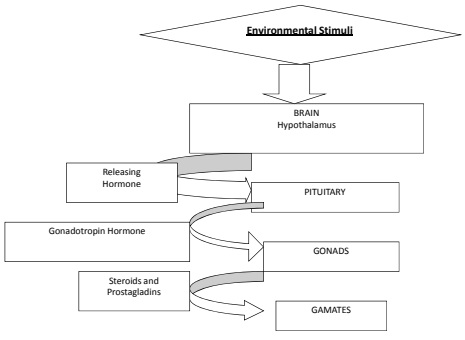
- Produced by crossbreeding channel catfish female with blue catfish male
- Is a genetic improvement program Performance = G + E + G x E
- Natural, Pen and Strip spawning are three methods to produce hybrid catfish
- Induced spawning and artificial fertilization technology to produce hybrid catfish

Hybrid Catfish Production



Hormone Induced Spawning of Catfish

- **3- day process**
- Day 1 : Select mature females – transport to hatchery – Weigh – Ist Injection (Evening)
- Day 2 : 2nd Injection (Morning); Select Blue catfish males; Prepare Hatching facilities
- Day 3 : Strip spawn ovulating females, Measure eggs, Add Blue catfish sperm – fertilize – Water harden eggs - Hatch



Processes involved in reproduction of fish

Lutenizing Hormone Releasing Hormone analog

- Synthetic peptide
- 1 mg; 5 mg; 25 mg
- Not species specific
- Higher level at BPG axis
- Short shelf-life
- Advances maturation
- Synchronizes ovulation
- Potency

Types of LHRHa

1. Mammalian LHRHa

D-Ala^6-Pro^9-Nethylamide-mLHRHa

Widely available, less expensive and effective in many fish species

2. Salmon LHRHa

D-Arg^6-Pro^9-Nethlyamid-sLHRHa

Less widely used, more expensive and more potent and used in few fish species

Brood fish selection

- Mature channel catfish (select older fish early in the season followed by younger fish) are hand selected that exhibit superior secondary sexual characteristics : soft and extended abdomen, fullness of the belly, pinkish and protruding vent.
- Transport the fish to the hatchery, weigh the fish (kg) and place them in a spawning bag

LHRHa Hormone Preparation

Recommended dose LHRHa: 100 ug/Kg
(20 ug/Kg + 80 ug/Kg)

100 kgs of broodfish (100 x 100 ug = 10,000 ug = 10 mg

Stock solution : Add 10 mL of saline in 10 mg LHRHa = 1000 ug/mL; saline is 0.85% salt solution)

Ist Injection (20ug/kg/mL)

Add 2 mL of stock solution + 98 mL of saline – 20ug/mL

2nd Injection (80ug/kg/mL)

Add 8 mL of stock solution + 92 mL of saline – 80ug/mL

LHRHa Hormone Injections

- Injection interval : 15 hours between 1st and 2nd injection
- The hormone must be mixed properly, labeled and stored for maximum potency.
- Use a 3 cc Syringe to inject the volume based on the weight (kg = cc) of the fish under the base of the pelvic fin. (Inject 1 kg fish with 1 cc of hormone solution, 1.5 kg fish with 1.5 cc)

Hormone Delivery Methods

- Delivery Methods
  - Intraperitoneal
  - Intramuscular
  - Intracranial
- Mode or Vehicle
  suspension / gel / implant
- Frequency
  - 1x, 2x

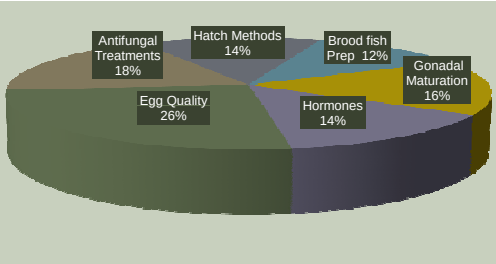
LHRHa injected channel catfish suspended in spawing bags for induced ovulation

LHRHa Field Trials in Hybrid catfish production

(2009)

Facility	Injected (#)	Ovulation (%)	Hatch (%)	Fry/Kg of Female
Hatchery1	393	54	23.8	796
Hatchery2	330	80	35.9	1757
Hatchery3	156	97.4	33.9	2000

Hybrid embryo production parameters



Spawning Bags

- 24" X 36 " Black Mesh bags with draw cord and closure (5/16 inch mesh size). It can be purchased from the vendor that is listed.
- Weigh the fish and place the fish in the bag, tie the bag with the draw cord and hung the bag in raceways or tanks.
- The fish should be suspended at least 6 inches below the water surface and placed between a foot between the bags.
- Place a plastic clip or tag to mark the bag for identification: know the weight of the fish for hormone injection or to identify the fish for ovulation.

Advantages of Spawning Bags

The present practice is to repeatedly handle the communally hormone induced fish for observation, leading to handling stress – reduces the performance.

Lack of knowledge of the latency time can

1) stripping the fish **too late** result in overripe eggs, leading to poor fertilization and hatch

2) stripping the fish **too early** result in early or immature eggs leading to no hatch

Checking for ovulation of LHRHa induced channel catfish in spawning bags

- At the end of the latency period (26 h at 82 F), slightly lift the spawning bag with fish above the water to see if eggs are deposited on the seam or on the bag.
- Mark or tag the bags to identify the ovulating fish. Then on, check the ovulating fish every hour or so.

Stripping the ovulating channel catfish

- Sedate the fish with the bag in an anesthetic solution.
- Remove the fish from the bag and rinse the fish in rinsing solution (161.5 g of non-iodized salt in 5 gallons of hatchery water).
- Wipe the fish with dry towels.
- Wrap the head and pectoral fins with the towel and expose only the ventral part of the fish.

Stripping the ovulating catfish

- Roll the fish on her back, holding the tail in your left hand and stroke gently with your right hand from head to vent to see a flow of eggs.
- Place pans or buckets that are slightly greased with vegetable shortening on a table.

Fertilizing the stripped channel catfish eggs

- Quantify the stripped eggs (measure volume or weight) and add blue catfish sperm solution (1 gram testes for 250 ml of eggs). Mix it with a plastic spoon.
- Pour the fertilized eggs (150 to 200 ml) in a series of greased cake pans
- Activate the fertilized eggs with a cup of hatchery water and gently stir and set it for a minute

Water hardening the fertilized hybrid catfish eggs

- Pour of the water gently without distrurbing the eggs.
- Slowly add hatchery water on the side of the pan without disturbing the eggs and fill it up with up water and allow it to settle for 10 min.
- Slowly flip the spawn in the pan and allow it to settle for 5 min.
- Transfer pans with developing spawns to a hardening tank, 4- 6 inch deep and supplied with water and air for 20 minutes.

Hatching the fertilized hyrbid catfish eggs in troughs

- Transfer the fresh spawn from the pan to hatching baskets that are suspended in a typical hatching trough.
- Place 2 or 3 spawns in a basket and do not crowd, as these spawn are going to swell the first 24 hours.
- The troughs are provided with continuous water and air and paddles to enable the fertilized eggs to hatch in 4 days (28C)

Considerations for hatching success of hybrid eggs

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Good and Bad strip spawned channel catfish eggs

Hatching of Catfish Eggs

- Natural Hatching in ponds
- Horizontal Hatching troughs
- Vertical Hatching troughs
- Sea-Saw Hatching
- Jar Hatching

Optimal needs of developing Hybrid catfish Embryos in the Hatchery

- Dissolved Oxygen (90 to 100% saturation)
- Temperature (80 to 82 F)
- Calcium needs (50 ppm)
- Water flow (2-3 flushes/hour)
- Constant Air and slow paddle movement
- Reduced or no crowding
- Reduced movement of embryos

Management Considerations to improve the production of hybrid embryos

- Handling and care of fertilized eggs
- Hatching baskets
- Swelling and shrinking of egg masses
- Removing dead eggs / fungus
- Immature eggs
- Diseases – Bacterial (Water temp > 82 F)
  - Fungus (Water temp < 78 F)

Chemical Treatment Guidelines

- Prevent shock
- Dose and length of treatment
- Effective treatment method
- Critical stage of embryo
- Approved Chemical

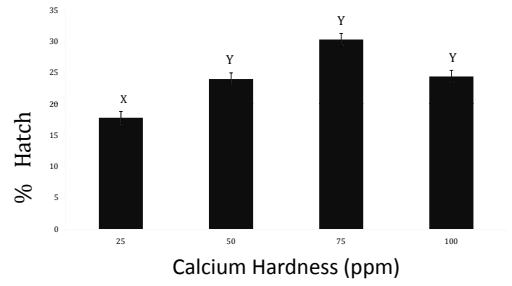
Chemical disinfectants to prevent fungal infections in hybrid eggs

- Povidone Iodine – 100 ppm
- Hydrogen peroxide – 250 ppm
- Formalin – 100 ppm
- Copper Sulphate – 2.5 ppm
- Salt 1 ppt

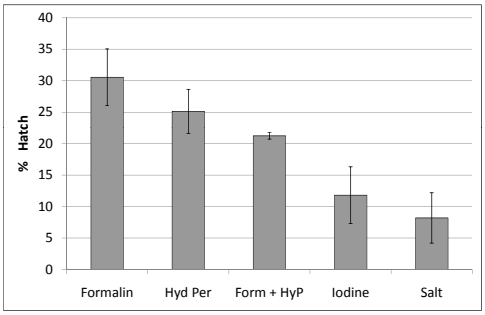
Differences in natural VS artificially spawned channel catfish egg

Criteria	Natural spawned	Artificially spawned
Age	Variable	Uniform
Condition	Good	Prone to damage
Maturation	Uniform & Complete	Variable and retained
Mating choice	Random	Selective
Chemotherapeutics	Recommended	Must
Hatching Space	Extensive	Manageable
Fertilization	0 – 100 % (80%)	0-100 % (80%)
Hatch	0-100 % (60%)	0 to 96% (40%)

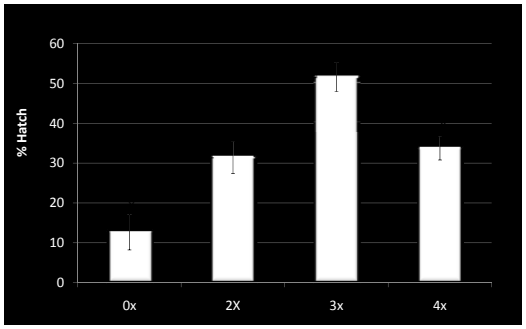
% Hatch (mean ± SE) of Hybrid catfish eggs incubated at 25, 50, 75 and 100 ppm of calcium hardness in replicated tanks



Hatching success of hybrid eggs treated with select chemical treatments at optimal levels during the course of their development



Hatching success of hybrid catfish eggs treated with 100 ppm formalin at a frequency of 0, 2, 3 and 4 times as a 15 minute interval bath



Effect of formalin treatments administered at 42 h post-fertilization (control) or withheld from 42-44, 42-46 and 42-48 h post-fertilization on hybrid hatching success at 28C